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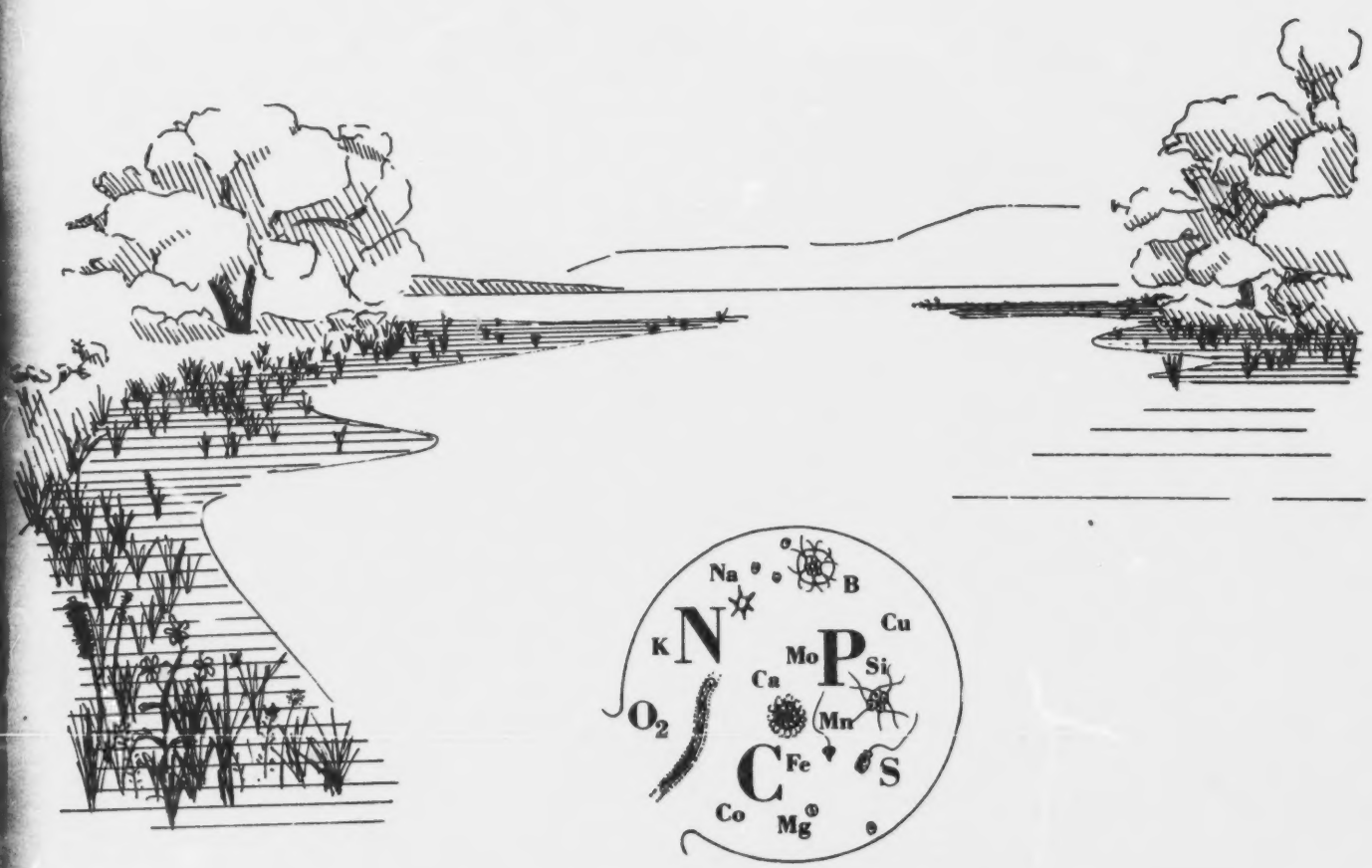
# Dissolved and Particulate Organic Carbon in Some Colorado Waters

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1. Report No. 2. W 72-10606

DISSOLVED AND PARTICULATE ORGANIC CARBON IN SOME COLORADO WATERS,

5. Report Date
6.
8. Performing Organization Report No.

Reed, E[redacted] B.

Colorado State Univ., Fort Collins, Dept. of Zoology

EPA Program
16010EQA 10/71

[redacted]

13. Type of Report and Period Covered

12. Sponsoring Organization

Environmental Protection Agency, Water Pollution Control Research Series 1[redacted] October 1971. 89 p. 19 fig., 28 tab., 20 ref.

Instantaneous amounts of organic carbon, both particulate and dissolved, in a number of freshwaters ranging from unproductive tarns to moderately productive reservoirs were measured by acid-persulfate digestion and infrared absorption. Organic carbon was designated as net seston carbon, filter seston carbon, or dissolved carbon based on filtering techniques. Concentrations of dissolved carbon ranged from about 1 to 11 g/cu m; filter seston amounts varied from about 0.2 to 1 g/cu m; the net fraction or organic carbon ranged from less than 0.1 to over 0.2 g/cu m. Repeated sampling showed amounts of organic carbon, either dissolved or particulate, fluctuated considerably over short periods of time; the dissolved fraction probably varying less than particulate forms. On incubation of all carbon fractions at room temperature in darkness for periods up to 90 days, concentrations of organic carbon varied erratically and unpredictably. Almost daily measurements in samples incubated over three week periods also revealed erratic changes, with no clear reduction in total organic carbon. Metabolic activities of heterotrophic bacteria (and algae?) probably complicated interpretation of results. The data have descriptive value, although they cannot, at this time, be related satisfactorily to the general limnological knowledge. (Jones-Wisconsin)

17a. Descriptors: *Carbon, *Distribution, Colorado, Lakes, Reservoirs, Streams, Detritus, Spatial distribution, Eutrophication, Laboratory tests, On-site investigations

17b. Identifiers: Organic carbon, Dissolved carbon, Particulate carbon

Copy available from GPO Sup Doc [redacted], $1.00 ; microfiche from NTIS as PB 210 705, $0.95.

05C, 02H

19. Security Class. (Report)
20. Security Class. (Page)
21. No. of Pages
22. Price

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# DISSOLVED AND PARTICULATE ORGANIC CARBON IN SOME COLORADO WATERS

by

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for the

Office of Research and Monitoring

ENVIRONMENTAL PROTECTION AGENCY

Project #16010 EQA

October 1971

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## ABSTRACT

Organic carbon in a variety of lakes, reservoirs and streams was measured by acid-persulfate digestion and infrared absorption.

Organic carbon was designated as net seston carbon, filter seston carbon or dissolved based on filtering techniques.

Concentrations of dissolved carbon ranged over an order of magnitude; i.e. from about 1 to 11 $g/m^3$; filter seston amounts varied from about 0.2 to 1 $g/m^3$; the net fraction of organic carbon ranged from less than 0.1 to over 0.2 $g/m^3$. In general bodies of water judged to be meso- to eutrophic contained more organic carbon than those waters tending toward oligotrophy.

Repeated sampling of selected bodies of water revealed that amounts of organic carbon, either dissolved or particulate, fluctuated considerably over short periods of time; however the dissolved fraction probably varied less than particulate forms of carbon.

Samples of all carbon fractions were incubated at room temperature in darkness for periods ranging up to 90 days. Concentrations of organic carbon varied erratically and unpredictably during incubation.

Almost daily measurements of carbon in samples incubated over three week periods also revealed erratic changes, with no clear reduction in total organic carbon. Metabolic activities of heterotrophic bacteria (and algae?) probably complicated interpretation of results.



This report was submitted in fulfillment of Project #16010 EQA under the sponsorship of the Water Quality Office, Environmental Protection Agency.

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# SECTION I

## CONCLUSIONS

Amounts of dissolved and particulate carbon in streams, lakes and reservoirs may fluctuate widely over short intervals of time. Dissolved organic carbon tended to be more uniformly distributed with depth in thermally stratified lakes than was the particulate fractions. The total amount of organic carbon seemed to correspond to the trophic status of the bodies of water investigated. That is, lakes judged to be the most productive contained the largest instantaneous amounts of organic carbon.

Under the conditions of this study, organic carbon did not decline uniformly or predictably in samples incubated in darkness at room temperature. The behavior of incubated samples could not be satisfactorily related to the apparent trophic status of the bodies of water from which samples were drawn.

## SECTION II

## RECOMMENDATIONS

The temporal and spatial distribution of dissolved and particular organic carbon should continue to be investigated in a number of waters covering a broad trophic spectrum. Inorganic carbon as well as organic should be monitored in order to better elucidate the movements of carbon in the freshwater environment. Such information is essential to evaluating the role of carbon in eutrophication.

Laboratory investigations of the mineralization of organic carbon during incubation should be conducted. Inorganic carbon should be monitored in order to stoichiometrically follow transfers within the total pool of carbon. Particular attention should be paid to the role of bacteria in incubated samples.

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## SECTION III

## INTRODUCTION

A large number of observations made in many areas and depths of the world's oceans have clearly revealed the presence of a sizeable fraction of detrital organic material that is refractory to bacterial breakdown. The absolute quantity for any geographic area appears to be remarkably constant and independent of local environmental conditions.

A parameter indicative of the degree of eutrophication of a body of water and insensitive to local or seasonal conditions would be of considerable value in detecting incipient pollution arising from unwanted eutrophication as well as theoretical importance.

The objectives of the research were 1) to measure instantaneous amounts of organic carbon, both particulate and dissolved, in a number of freshwaters ranging from unproductive alpine tarns to moderately productive reservoirs on the plains; 2) to determine if a fraction of particulate organic carbon refractory to bacterial decomposition could be demonstrated and 3) to relate refractory or nonrefractory organic carbon to trophic levels of the waters studied.

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## SECTION IV

## METHODOLOGY AND PROCEDURE

Organic carbon is, for the purposes of this investigation, divided into three categories based on particle size: 1) net seston organic carbon (NSC) - particles retained by plankton netting of 70 nm mesh; 2) filter organic carbon (FSC) - particulate carbon passing the 70 nm net, but retained on glass fiber filters, i.e. lower limit of about 1 - .5 nm; 3) dissolved organic carbon (DOC) - carbon in solution or particles fine enough to pass through glass fiber filters.

Field Sampling - Samples of water and included seston were obtained with either a Kemmerer or Van Dorn sampler. Rental boats are available at Horsetooth Reservoir near Fort Collins; an inflatable rubber raft was packed to remote lakes. Vertical distribution of carbon was assessed by taking samples at each 1-m interval from surface to near bottom.

In order to reduce variability, samples were integrated by combining adjacent probes according to this scheme: in the uppermost 10-m stratum immediately adjacent samples were combined, i.e. 1 and 2 m; 3 and 4; 5 and 6 etc. Between depths of 11 and 25 m, three adjacent samples were composited, i.e. 11, 12, and 13 m; 14, 15 and 16 m, etc. At depths exceeding 25 m, compositing was done by 4 m intervals, i.e. 26, 27, 28, and 29 m, etc.

Vertical profiles of temperature were usually obtained at each sampling site.

Numerous investigations of sestonic and dissolved carbon have clearly indicated that the dissolved fraction is substantially greater than the particulate (i.e. Birge and Juday 1934). In order to secure sufficient NSC for reliable analyses and simultaneously reduce the amount of water to be transported from the field to the laboratory, NSC was strained from the water at the time of sampling. Strainers were made by cutting the bottoms from polyethylene bottles of 1000 and 500 ml capacities. Holes large enough to remove most of the ends were drilled in the caps; discs of 70 u netting were cut to fit snugly within the caps.

Two to 12 liters of water could be strained before clogging of the net became a serious problem, thus yielding sufficient net seston for analyses.

From the water passing through the strainer, 250 to 1000 ml samples were retained for FSC and DOC fractions and taken to the laboratory.

Laboratory Procedures - Samples were passed through a Millipore vacuum filtering device fitted with Whatman GFC glass fiber filters. Known volumes of water were filtered; amounts varied according to amount of seston present. Filtering stopped when vacuum greater than 4 psi was required to overcome clogging of filters. Water passing glass fiber filters was saved for DOC determinations and some chemical analyses



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(i.e. hardness, calcium, $NO_3$, $PO_4$, and $SiO_2$). Chemical analyses were incidental to the main objectives of the investigation and will not be discussed in the present report.

Menzel and Vaccaro (1964) developed a technique for determining organic carbon. Their procedures with slight modification, to be discussed later, were followed. In essence, organic carbon is digested with persulfate and converted to gaseous carbon dioxide. The gas is passed through a Beckman infrared analyzer where infrared absorption by the $CO_2$ is determined. Inorganic carbon present as $CO_2$ from the atmosphere or as $HCO_3$ is removed by acidification and stripping with nitrogen prior to persulfate digestion. Digestion is accomplished in sealed glass ampoules autoclaved for 40 min at 31 psi.

For DOC, 5 ml of filtered lake water was introduced directly into 10 ml ampoules, heated and analyzed.

Net seston was divided into aliquots in the laboratory, one for immediate analysis, the others to be analyzed after varying periods of incubations. Aliquots of net seston were suspended in 5 ml of distilled water. Analyses proceeded as for DOC.

It was planned to make aliquots of FSC as was done with NSC. Filters as received from the manufacturer are contaminated with carbonaceous compounds that influence analyses. Menzel and Vaccaro (1964) recommended precombusting filters to remove carbon contamination. The only furnace available at the outset of this study melted filters when set at its lowest temperature. Wet oxidation using sulfuric acid and potassium dichromate was tried, but proved unsatisfactory. Filters were assayed for carbon in the hope that a "blank" or correction factor could be obtained. Wide variation in results dashed this hope; therefore, no determinations of FSC were made prior to 1 January 1971.

The infrared analyzer was calibrated by preparing solutions of dextrose containing 2.5, 5.0, 10.0, 20.0, 40.0, 1000.0 and 200 mg C/liter. Series were prepared by serial dilution of a concentrated stock solution. New stock solution was made for each series of calibration standards. Calibration series overlapped - i.e. each new series was checked against the previous series.

Before unknown samples were analyzed, the machine was set to give 100% scale deflection at a known carbon value i.e. for routine determination of DOC 10 mg C/liter; heavy net seston sometimes required that the machine be set at 40 or even 100 mg C/liter.

After setting, not less than three different standards were analyzed, i.e. with the analyzer set on 10 mg C/liter, samples of 10, 5 and 2.5 mg C/liter were used in addition to distilled water. From the calibration run a least squares regression line relating carbon to percentage of deflection on scale was computed. Unknowns were then analyzed and

percentages of deflection converted to carbon values from the regression line.

Methodological Modifications - Menzel and Vaccaro (1964) cautioned that ampoules must be sealed in an oxyflame to guard against contamination from combustion products. At first, ampoules were sealed in an ordinary Bunsen burner flame since oxyequipment was not immediately available. Combustion contamination was at once revealed by erratic and unpredictable values using known concentrations of dissolved dextrose and distilled water. After oxyflame equipment became available, no further trouble of this sort was experienced.

Menzel and Vaccaro (1964) recommended Mg $(ClO_4)_2$ as an agent to dry the gas stream immediately prior to entering the infrared analyzer. We substituted indicating Drierite for Mg $(ClO_4)_2$ with excellent results. Drierite was changed as soon as it changed from blue to pink in the upper one fourth of the drying tube.

Strickland and Parsons (1968) in their discussion of Menzel and Vaccaro's method pointed to the importance of organic-free water. Following their suggestions distilled water containing potassium persulfate and phosphoric acid was refluxed prior to redistilling in all glass apparatus.

Because Menzel and Vaccaro (1964) studied carbon dissolved in sea water, they were concerned with chlorine contamination, resulting from persulfate action on sea water. They passed the $CO_2$ - Nitrogen stream through a washing bottle containing potassium dichromate dissolved in sulfuric acid. Chlorine contamination seemed unlikely, at least in most of the waters to be investigated; therefore, an experiment testing the effect of removal of the gas washing bottle was performed. A least squares regression was determined from water containing 0, 2.5, 5.0, and 10.0 mg C/liter with the washing bottle in the instrument train; four replicate samples of Dixon Reservoir water were then analyzed. The wash bottle was removed and the entire process repeated. Least squares regression equations were, with bottle in place, $Y=7.42X + 28.30$; with bottle removed, $Y=7.07X + 26.30$. Apparently the wash bottle did not enhance performance (Table 1) and thus has been removed from routine use.

Sensitivity of Method - The ability of the method to detect low concentrations of dissolved carbon was evaluated by preparing replicate samples containing 0, 1.0, 2.0 and 3.0 mg C/liter. Absolute amounts of dissolved carbon in 5 ml ampoules were, 0, .005, .010 and .015 mg respectively.

Because 95% confidence intervals did not overlap (Table 2), we concluded that the analyses could detect the differences in carbon concentrations. A regression line was established with 10 mg C/liter standards producing full scale deflection or 100% absorbance. Carbon values were then picked from the regression line using percentages obtained from sensitivity study (Table 2). Means and ranges were 0 mg C/liter, .35 mg, 0.-.75;

1 mg C/liter, 1.40, 1,05-1.75; 2 mg C/liter, 3.0, 2.40-3.45 and 3 mg C/liter, 5.5, 4.45-6.60.

Clearly the use of a regression line to convert percentages of absorption to carbon concentration introduces problems that are distinct from those associated with accuracy and precision of detection of carbon in unknown samples.

Accuracy of Method - It is possible to calibrate the analyzer against weighed amounts of carbon, but does the method convert all organic carbon present to carbon dioxide? Insight to this problem was gained by adding a weighed amount of urea to filtered water taken from Horsetooth Reservoir. Eight replicates were analyzed; eight replicates of Horsetooth water lacking the urea spike were also analyzed (Table 3). The analyzer was adjusted so that 10 mg C/liter, as dextrose, produced full scale deflection. Mean carbon content of unspiked water was 3.20 mg C/liter and that of the spiked samples was 6.35 mg C/liter. The spike consisted of 15.9 mg urea in one liter of water, thus $\frac{15.9}{1} \times \frac{12.0}{60.6} = 3.1485$ mg C. The mean value of spiked samples, 6.35 minus 3.15, carbon added as urea equals 3.20, the mean value for unspiked samples.

Undoubtedly, 100% recovery of added carbon is fortuitous, but low coefficients of variability are reassuring.

Sensitivity of analyses and method was evaluated by diluting lake water with distilled water (Table 4). Water from Cub Lake, a small brown-water montane lake, was filtered, diluted and analyzed with the machine calibrated at 10 mg C/liter.

Non-overlap of confidence limits suggests that the method and machine discriminated among 0, one-half and two-thirds dilutions. Percentage of error (SE X 100/Y) increased with increasing dilution, namely 1.3, 2.1 and 4.9%.

The machine was recalibrated at 20 mg C/liter; filtered Cub Lake water, full strength and 50% diluted, was re-analyzed. Two determinations of undiluted water yielded identical results, 8.8 mg C/liter. Two analyses of 50% dilution gave a mean of 5.2 mg C/liter with a 95% limit of 2.66-7.74, or nearly 40% error.

Filter Seston Carbon - After unsuccessful attempts to either decontaminate filters or find a suitable correction factor, we tried analyzing filtered and unfiltered lake water, hoping to estimate FSC by difference.

Three replicates of unfiltered Cub Lake water were compared with two replicates of filtered water with the carbon analyzer calibrated at 20 mg C/liter. Identical values of 8.8 mg/liter were obtained from filtered samples. Mean of unfiltered samples was 9.2 mg/liter, but the 95% limits were 8.3-10.1, thus suggesting insignificant difference. Percentage of error for three unfiltered samples was 2.3%.

Similar experiments were done using water from Dixon Reservoir with the analyzer calibrated on 10 mg C/liter (Table 5).

Most likely these differences are not significant. The recorder scale is probably not accurate to more than ± one small division, i.e., 1% of full deflection. Because the largest value is only 2 percentage points greater than the smallest, machine error could account for differences.

Rupture of algal cells during filtration could, however, cause abnormally high values of DOC and simultaneously diminish FSC. Rupture of fragile cells is a problem frequently encountered in C-14 uptake assays of primary production rate (Vollenweider, 1969).

Experiments were repeated with Dixon Reservoir water and the analyzer calibrated at 5 mg C/liter. Three replicates of unfiltered water each gave 115% absorbancy while three filtered values were 115, 115 and 120%. All values were determined by extrapolation, a dangerous procedure since one cannot assume linearity when changing scales on the analyzer.

Cub Lake and Dixon Reservoir were chosen because previous studies (Reed and Reed 1970 and unpublished) indicated substantial standing crops of nannoseston in their waters. The method of difference is not satisfactory for distinguishing FSC from DOC in our procedure.

Incubation - One of the objectives of the study was to follow the release of carbon during incubation of samples in the laboratory. At first, breakdown of DOX was followed in 300 ml BOD bottles, later 60 ml reagent bottles of filtered lake water were stored in dark boxes at room temperature for periods lasting to 90 days. Net seston was incubated in distilled water. One possible objection is that bacteria may have become nutrient limited thus producing unnatural rates of breakdown of organic matter. Therefore, replicate samples of net seston obtained from Horsetooth Reservoir were divided into three lots, one for immediate carbon analysis, two to be incubated. One lot of incubated samples was treated with $Ca(NO_3)_2$ at 5 mg/liter. Because this experiment illustrates a number of problems encountered in incubation studies, it will be described in detail.

Several liters of reservoir water were obtained and thoroughly mixed in a large container in the laboratory. One-liter subsamples were drawn, with continuous stirring. Each subsample was strained through 70 u plankton netting. The catch was carefully rinsed into 10 ml ampoules with 5 ml of distilled water and treated in the routine manner.

The infrared analyzer was calibrated at 20 mg C/liter (0.1 mg C produced full scale deflection). A regression line was determined using standards of 20, 10, 5, and 2.5 mg C/liter and distilled water (0 carbon). Six replicates were then analyzed.

Twelve more replicates were drawn and strained in the same manner; to six, $Ca(NO_3)_2$ was added. Each ampoule was covered with a cap of aluminum foil. Samples were incubated in a dark box at room temperature, 22C$\pm$ 3C for nine days. They were then analyzed for carbon content after calibrating the analyzer with 20 mg standards. A new regression line was determined.

Thus, this experiment involved not only the possible effects of the nitrate on incubation but a number of other sources of variation: 1) subsampling, 2) possible loss of seston in straining, 3) contamination of distilled water used in black, 4) standards and 5) inherent in machine.

Before discussing results of this particular experiment, it should be emphasized that it was done rather early in our experiences with the analyzer and procedure. Later the analyzer was allowed to run continuously, thus eliminating warm-up problems which may have influenced earlier results. Reproducibility of standards received constant attention and, as far as can be determined, was well within other sources of error.

Despite repeated efforts including double glass distillation we were unable to achieve zero absorbance from distilled water blanks. Examination of a graph of absorbance vs. carbon content given by Menzel and Vaccaro (1964) revealed that their regression line did not pass through the origin but gave a positive intercept on the ordinate, indicating absorbance from supposedly carbon free water.

Distilled water that would yield zero absorbance was not achieved, although following the procedure of Strickland and Parson (1968) "carbon-free" water that regularly reads less than 10% absorbance, usually less than 5% was obtained. Thus, regression equations were influenced by different values for supposedly carbon free water. The regression equation for the control series of samples, those analyzed immediately upon collection, was $Y = 4.40 X + 13.64$. Mean carbon content was .033 mg C per liter of reservoir water strained (Table 6).

After nine days, the incubated samples were analyzed; new regression equation was $Y = 4.10 X + 20.50$. Mean of enriched samples was .030 mg C/liter and that of unenriched samples was .037 (Table 6).

These data indicated no significant difference among control and incubated samples at the 95% level. Confidence levels of control and unenriched incubation samples did not overlap at 70% level.

A new regression equation was derived using blanks and standards of the two separate regressions and percentages of absorbance reconverted to carbon (Table 7). Results were not noticeably improved. Means retained the same relative rankings, but amounts were nearly halved. Results were analyzed using raw absorbance percentages; thus eliminating regression equations (Table 8).

The mean of unenriched incubated samples apparently differed significantly at 70% level from the means of the other samples. Omitting conversion of absorbance to carbon slightly reduced percentages of error, but did not alleviate the seeming problem of incubated means exceeding the control. Perhaps nine days incubation was too short a period for significant differences to develop; however, the difficulties of obtaining exactly the same regression equations at different times are illustrated.

Ways in which incubated samples could gain significant amounts of carbon during incubation will be considered in the discussion of results of other incubation experiments.

Difficulties in securing large amounts of seston and in storing and handling samples in the laboratory discouraged multiple sampling of seston. Therefore, incubation of NSC samples was not run in duplicate; that is, only one sample represented any given depth over a particular time interval. In contrast, rather large volumes of water for DOC determinations could be readily obtained and stored.

Presumably DOC would change little in 24 hours after having been incubated for 15 days, thus offering the possibility of checking reproducibility of machine operation and laboratory procedures.

Samples of Blue Lake water representing various depths were analyzed after 15 days of incubation. On the next day a second series was run. The machine was recalibrated for the second trial and two separate regression lines determined for converting absorbancy to mg C/liter. There was no significant difference in means of the two trials (Table 9). High variability indicated that DOC was not evenly distributed in the water column. From a methodological point of view, it is gratifying that the samples retained their rankings relative to each other.

Variability Among Replicate Samples - The variability in carbon content among replicate samples of net seston was evaluated by drawing a quantity of water from Dixon Reservoir and transporting it to the laboratory where, with continuous stirring, five aliquots were taken for carbon analysis. The analyzer was calibrated so that .50 mg C (100 mg C/liter) produced full-scale deflection; regression equation was $Y = .84 X + 22.08$. Results are summarized in Table 10.

Percentage of error in NSC determination seems high, but perhaps not excessively so when possible sources of error are considered; for example, in drawing subsamples, loss in straining and washing seston into ampoules and problems inherent in large particles, eight large Daphnia cannot be evenly distributed among five ampoules.

Table 1. Effect of gas washing bottle in instrument train, values as percentage absorption

| | Bottle in place | Bottle removed |
|---|---|---|
| n | 4 | 4 |
| $\bar{Y}$ (%) | 74.75 | 71.62 |
| $S^2$ | 21.43 | 2.21 |
| s | 4.63 | 1.49 |
| SE | 2.31 | .74 |
| 95% limits | 67.40 - 82.10 | 71.38 - 71.86 |
| error | 3% | 1% |
| CV | 6% | 2% |

Abbreviations used in this and subsequent tables:

n, number of observations; $\bar{Y}$, mean of observations

$S^2$, variance; s, standard deviation

SE, standard error $s/\sqrt{n}$; 95% limits, fiducial limits

P=.95; error, 100 $SE/\bar{Y}$; CV, 100 $s/\bar{Y}$

Table 2. Detection of dissolved carbon in low concentrations, percentage absorption

| Concentration, mg. C/liter | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| n | 7 | 8 | 10 | 10 |
| $\bar{Y}$ (%) | 27.36 | 34.81 | 46.95 | 65.80 |
| $s^2$ | 7.727 | 8.924 | 36.914 | 125.678 |
| s | 2.780 | 2.987 | 6.076 | 11.210 |
| SE | 1.051 | 1.056 | 1.921 | 3.545 |
| 95% limits | 24.79- | 32.31- | 42.60- | 57.78- |
| | 29.93 | 37.31 | 51.30 | 73.82 |
| error % | 4 | 3 | 4 | 5 |
| CV | 10 | 10 | 13 | 19 |

Table 3. Analyses of Horsetooth Reservoir water with and without addition of urea

| | Spiked | Unspiked |
|---|---|---|
| n | 8 | 8 |
| $\bar{Y}$ absorbance | 71.56 | 47.00 |
| $s^2$ | 12.60 | 10.00 |
| s | 3.55 | 3.16 |
| SE | 1.26 | 1.12 |
| error % | 2 | 2 |
| CV | 5 | 7 |
| 95% Confidence limit, absorbancy | 68.59 - 74.53 | 44.36 - 49.64 |
| 95% limits, carbon mg/liter | 5.95 - 6.70 | 2.85 - 3.50 |

Table 4. Dissolved organic carbon in Cub Lake water following dilution with distilled water

| | percentage dilution | | | |
|---|---|---|---|---|
| | 0 | 50 | 66 2/3 | 83 1/3 |
| n | 2 | 3 | 3 | 1 |
| $\bar{Y}$ mg C/liter | 7.95 | 4.22 | 2.63 | 1.25 |
| $S^2$ | .02 | .03 | .05 | -- |
| s | .14 | .16 | .23 | -- |
| SE | .10 | .09 | .13 | -- |
| 95% limits | 6.68-9.22 | 3.82-4.62 | 2.07-3.19 | -- |
| error % | 1 | 2 | 5 | -- |
| CV % | 2 | 4 | 9 | -- |

Table 5. Carbon determinations of Dixon Reservoir water

| unfiltered | | filtered | |
|---|---|---|---|
| percentage absorbance | carbon mg/liter | percentage absorbance | carbon mg/liter |
| 77.0 | 7.05 | 78.5 | 7.20 |
| 76.5 | 7.00 | 78.0 | 7.15 |
| 78.5 | 7.20 | 78.5 | 7.20 |
| mean | 7.08 | | 7.18 |

Table 6. Analyses of net seston incubated with and without addition of nitrate, separate regression

| | Initial | Enriched | Unenriched |
|---|---|---|---|
| n | 6 | 6 | 6 |
| $\bar{Y}$ (mg C/liter) | .0331 | .0302 | .0368 |
| $S^2$ | .0000383 | .0000297 | .0000543 |
| s | .00619 | .00544 | .00736 |
| SE | .00277 | .00244 | .0033 |
| 95% limits | .0260-.0402 | .0239-.0365 | .0283-.0452 |
| error % | 8 | 8 | 9 |
| CV | 19 | 18 | 20 |

Table 7. Analyses of net seston incubated with and without addition of nitrate, combined regression

| | Initial | Enriched | Unenriched |
|---|---|---|---|
| n | 6 | 6 | 6 |
| $\bar{Y}$ mg C/liter | .0151 | .0162 | .0199 |
| $S^2$ | .0000104 | .0000049 | .0000122 |
| s | .00322 | .00221 | .00349 |
| SE | .00144 | .00099 | .00156 |
| 95% limits | .0114-.0188 | .0137-.0187 | .0159-.0239 |
| error % | 10 | 6 | 8 |
| CV % | 21 | 14 | 18 |

Table 8. Analyses of net seston incubated with and without addition of nitrate, raw percentages of absorbance

| | Initial | Enriched | Unenriched |
|---|---|---|---|
| n | 6 | 6 | 6 |
| $\bar{Y}$ (%) | 42.75 | 44.58 | 50.66 |
| $s^2$ | 29.67 | 12.84 | 36.57 |
| s | 5.48 | 3.72 | 6.05 |
| SE | 2.44 | 1.66 | 2.70 |
| 95% limits | 36.49-49.01 | 40.30-48.86 | 43.71-57.61 |
| error % | 6 | 4 | 5 |
| CV % | 13 | 8 | 12 |

Table 9. Analyses of Blue Lake DOC after 15 and 16 days of incubation in the laboratory

| depth | day 15 | day 16 |
|---|---|---|
| 1-2 m | 1.90 mg C/liter | 2.15 mg C/liter |
| 3-4 | 1.30 | 1.55 mg C/liter |
| 5-6 | 1.10 | 1.25 mg C/liter |
| 7-8 | 3.30 | 3.25 mg C/liter |
| n | 4 | 4 |
| $\bar{Y}$ (mg C) | 1.90 | 2.05 |
| $S^2$ | .987 | .780 |
| s | .993 | .883 |
| SE | .573 | .510 |
| 95% limits | .32-3.48 | .64-3.45 |
| error | 30 | 25 |
| CV | 52 | 43 |

Table 10. Carbon content in replicate samples of net seston, Dixon Reservoir, 29 September 1970

| | |
|---|---|
| n | 5 |
| $\overline{Y}$ mg/liter | .352 |
| $S^2$ | .0303 |
| s | .1741 |
| SE | .0778 |
| 95% limits | .1360-.4880 |
| error % | 22 |
| CV % | 49 |

## SECTION V

## FIELD AND LABORATORY STUDIES PRIOR TO 1 JANUARY 1971

Dissolved Organic Carbon - The results of this investigation contain two sorts of data, namely, observations on the instantaneous concentrations of organic carbon at various depths in several different kinds of water bodies, and secondly, on changes in organic carbon concentrations after varying periods of incubation. Since incubation studies must include instantaneous values at moment of sampling, it is convenient to present results of incubation studies first. $T_0$ always refers to date of sampling, $T_{15}$, $T_{30}$, $T_{60}$ and $T_{90}$ refer to 15, 30, 60 and 90 days after $T_0$. In the tables to follow, values are usually the average of two, sometimes three determinations or, occasionally only one determination.

Mean value of dissolved organic carbon in water column at Station A on Horsetooth Reservoir, a large mesotrophic body of water at an altitude of 1656 m, ranged from 2.48 mg/liter to 6.29; from 67 to 82% remained after 90 days incubation (Tables 11-14). Mean carbon fell in first 15 days of incubation in two cases and in two others rose to a value higher at $T_{30}$ than at $T_0$ (Figure 1).

A series of samples was obtained over the deepest water in Bear Lake, a montane lake, on 30 July 1970 for incubation (Table 16). After 90 days, the mean carbon content was reduced from 5.30 mg/liter to 2.47, or a reduction of 53%. A rapid loss of carbon occurred during the first 15 days of incubation; little change took place in the next 15 days, but carbon increased to 82% of the original value at the end of 60 days. During the last 30 days, carbon content declined rapidly. (Figure 3).

Samples were obtained from Cub Lake, a brown-water montane lake, 30 July 1970 (Table 17). After 90 days of incubation mean DOC content had decreased 49% (Figure 4).

Two alpine lakes (Tables 18 and 19) were sampled on 11 August 1970. In general, mean DOC decreased in the first 15 days of incubation; composite samples from three depths in highly oligotrophic Green Lake exhibited similar trends in loss of DOC (Figure 5). Samples from five depths in Blue Lake behaved dissimilarly and, after 90 days, mean loss of DOC was only 6% (Figure 6).

Observed extreme values of DOC at Station A, Horsetooth Reservoir ranged from 1.50 to 8.20 mg/liter. Early in the summer maximum values were found in the upper 2 meters (Figure 9). In general, DOC increased in the water column as summer progressed to autumn and on 17 October exhibited a bimodal distribution. DOC apparently collected in the hypolimnion (Figure 9) and in the epilimnion.

Details of the vertical distribution of DOC in the upper 14 meters of Horsetooth Reservoir at Station C on one occasion are shown in Table 15. The epilimnion extended down to 9 m and metalimnion ranged from 10 to 14 m.

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Spatial differences in DOC in Horsetooth Reservoir are suggested by the data of Tables 12 and 15. Stations C and A are about 1 km apart, but Station C is in the flow of the inlet; whereas Station A is not in the direct flow through the reservoir. The stations were sampled about one week apart. Little difference was observed in the upper 10 m, but from 11-14, Station C contained about 1 mg C/liter less than Station A. Apparently the amount of DOC in one body of water can vary considerably over short periods of time, Dixon Reservoir, a small reservoir near Horsetooth Reservoir, was sampled on five occasions between 7 August and 29 September. All samples were drawn from a depth of 1 meter. DOC ranged from 3.98 to 7.35 mg/liter (Figure 2).

Elsewhere DOC ranged from about 1.20 to 2.50 mg/liter in Green Lake; 1.42 to 4.40 in Blue Lake; 3.90 to 5.98 in Bear Lake and was about 8 mg/liter in Cub Lake.

Net Seston Carbon - Because NSC comes from rather large particles which are often unequally distributed among subsamples, results are expected to be more variable than in DOC, which, in theory at least, is equally distributed within one water sample. Losses of samples and other laboratory malfunctions further reduced the number of useable results arising from incubation studies of net seston.

Net seston samples were obtained at Station 2, Horsetooth Reservoir on 15 July and 13 August 1970 (Table 20). Initial values ranged from .0303 mg C/liter (9-10 m, 13 August) to .0020 mg C/liter (35-37 m, same date). Mean NSC value on 15 July was .0117 mg/liter; this increased to .0130 mg/liter on 13 August. After 60 days of incubation, eight samples contained a mean of .022 mg C/liter or 188% initial value.

The series of 9 September (Table 21) and 17 October (Table 22) are more complete. Extreme initial values ranged from .157 mg C/liter (1-2 m, 17 October) to a low of .0082 (38-41 m, 9 September ). The mean values for NSC during incubation ranged from 94 to 162% of initial value for the 9 September series (Figure 7). Initial mean value for the 17 October series was much greater, but fell to 55% of the original value after 90 days.

Net seston from Dixon Reservoir was not incubated in the first half of this investigation; the reservoir was, however, repeatedly sampled to gain insight into changes in NSC. Between 15 August and 15 October 1970, the amount of NSC varied by an order of magnitude, the largest value being 12 times the smallest (Figure 8).

Within 30 days, the mean NSC of Bear Lake samples collected 30 July was reduced 91% (Table 23). A series of NSC samples drawn from Blue Lake on 3 September averaged slightly less in carbon and lost 93% after 60 days incubation. Carbon in net seston taken from Green Lake 3 September averaged slightly over one-half as much as Blue Lake and ultimately lost about the same percentage after 60 days of incubation (Table 23).

At Station A in Horsetooth Reservoir, NSC varied from .002 to .157 mg/liter. Depth and time trends (Figure 10) are not easy to summarize; NSC did increase through the summer and usually the maximum was found at some depth shallower than 10 m. Bear and Blue lakes usually showed about .04 mg NSC/liter and Green Lake about half of that amount (Table 23). NSC values in Dixon Reservoir ranged over an order of magnitude in two months time, 0.029 to 0.352 mg/liter (Figure 8).

The relationship of NSC to dry weight of net seston was examined on several occasions. Vertical profile of NSC vs dry weight was examined at Station A, Horsetooth Reservoir, 17 October; carbon was 10% of dry weight of seston (Table 26).

NSC vs dry weights of seston was examined on two different occasions in Dixon Reservoir; during a bloom of Aphanizomenon, mean weight of carbon was 16% of mean dry weight (Table 25). On 12 October when seston dry weight was nearly double that on 29 September, carbon was less than 1% of dry weight. These percentages are low for carbon; Kerr, Paris and Brockway (1970:) reported that carbon accounts 50-77% of dry weight of algae and about half of the dry weight of bacteria. Reed and Reed (1970) showed that inorganic materials compose a large part of the seston in Dixon Reservoir.

Samples were taken from Bear Lake 23 September and analyzed for NSC, dry weight of NS and DOC by depth strata. On the average NSC was only 4.4% of the seston dry weight (Table 24) and DOC was 344 times greater than NSC.

Observations of DOC in Streams - DOC was measured in Spring Creek, a plains stream near Fort Collins, on four occasions. Mean values at eight stations along the 10 km length of the stream ranged from 1.22 to 13.8 mg/liter (Figure 11). On 7 August the Cache la Poudre River was sampled at seven locations ranging from 10 km upstream from Fort Collins to 6 km downstream from the city limits. Eleven determinations varied between 3.35 and 4.55 mg DOC/liter. Effluent from a tertiary sewage treatment plant contained 7.48 mg DOC/liter, where it entered the Poudre River.

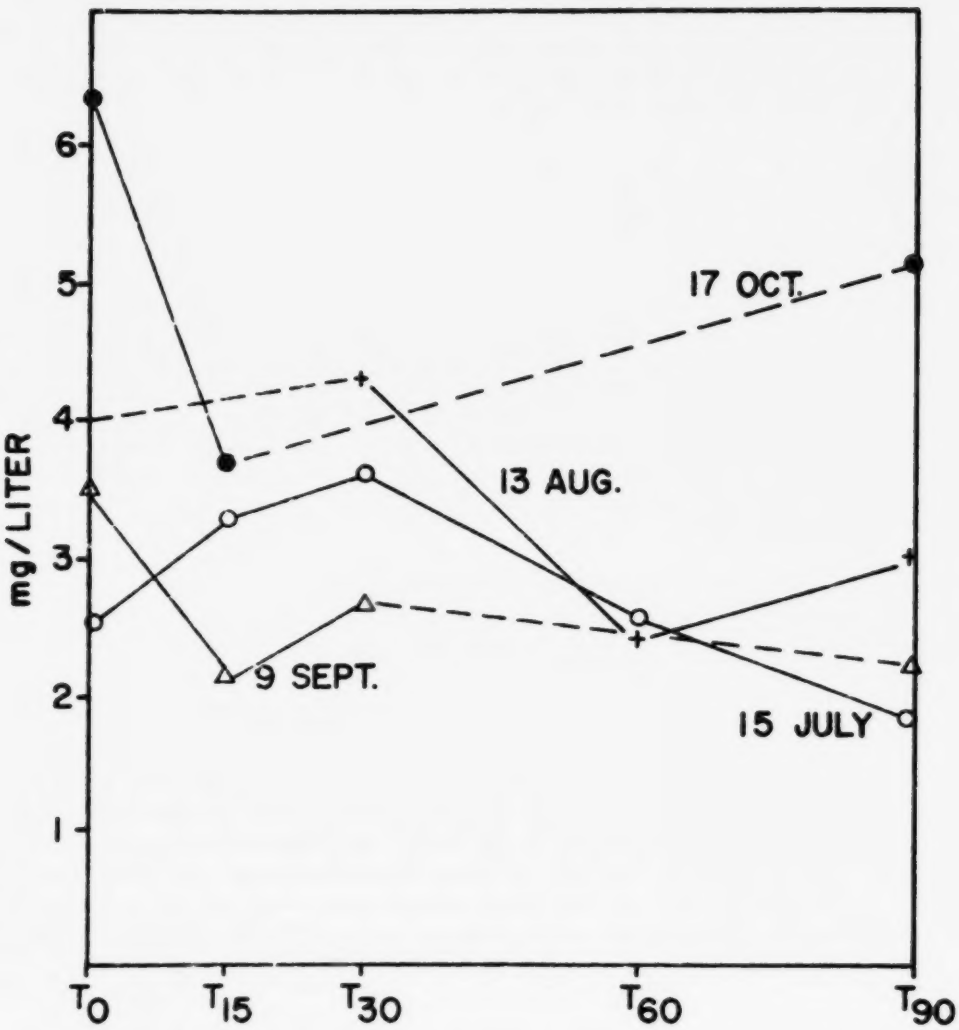


Figure 1. Mean dissolved organic carbon in water column, Station A, Horsetooth Reservoir on four dates and after incubation.

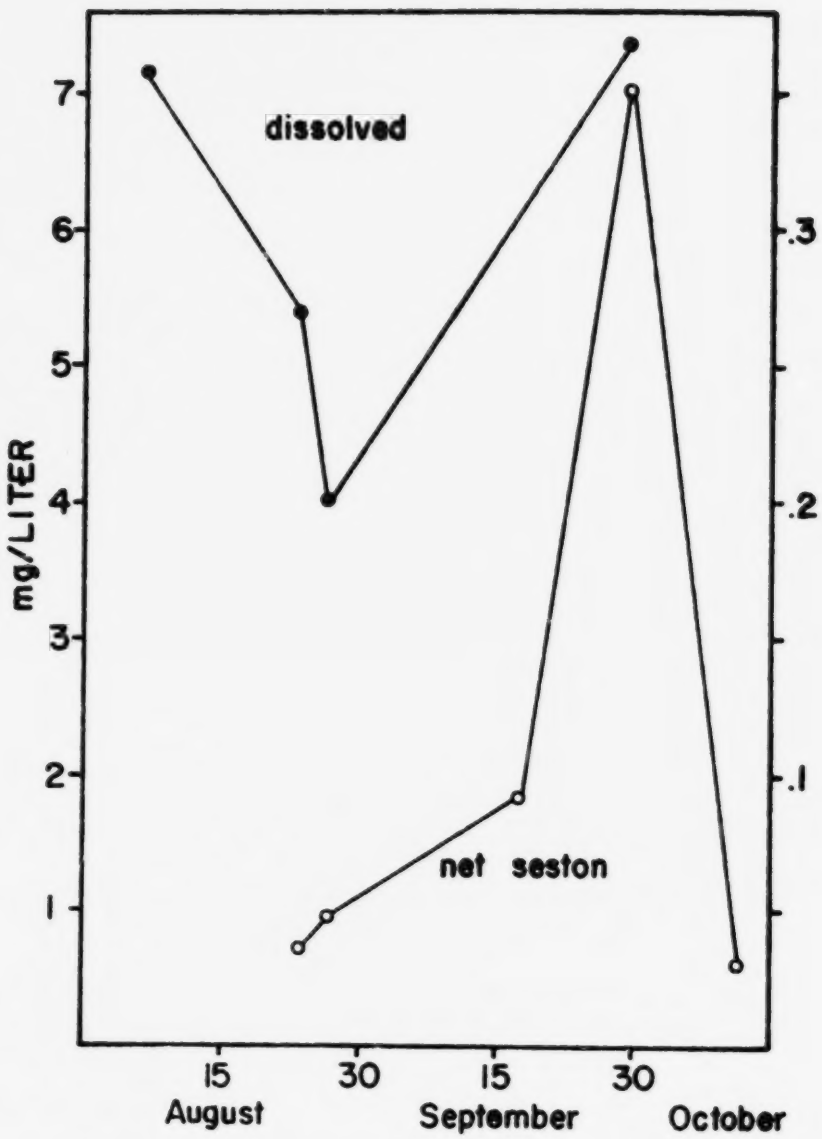


Figure 2. Dissolved organic carbon (left ordinate) and net seston carbon, Dixon reservoir.

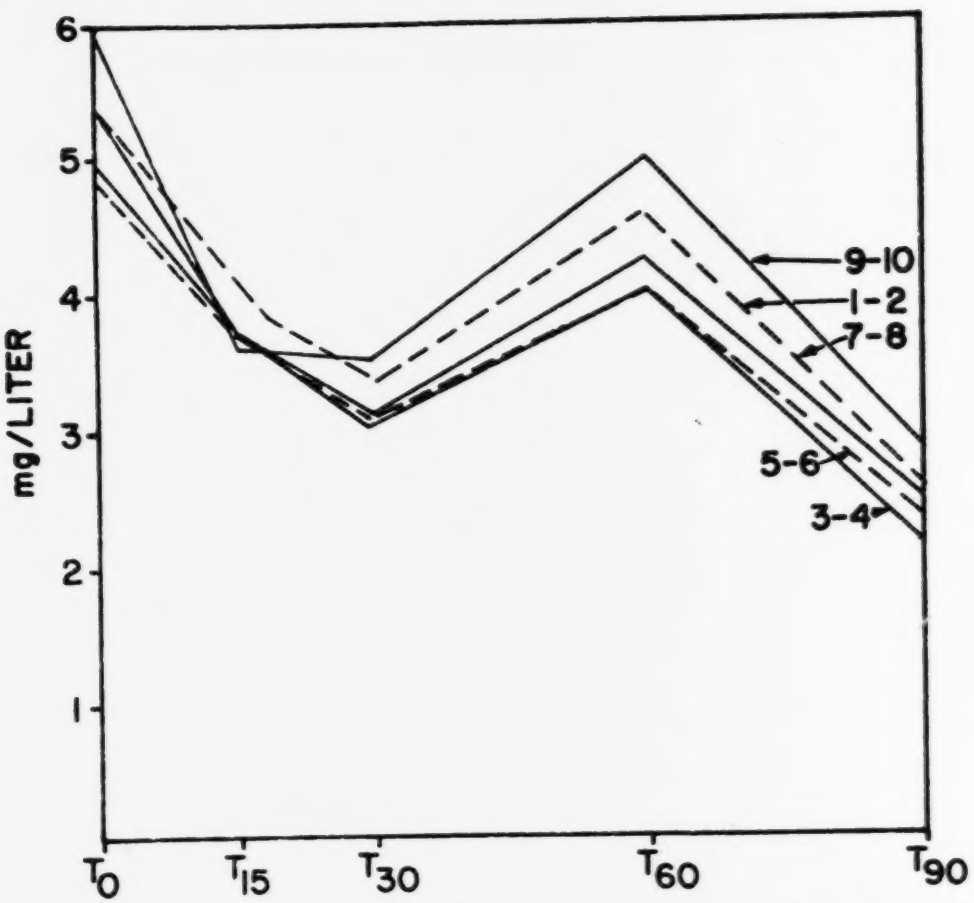


Figure 3. Vertical distribution of dissolved organic carbon in Bear Lake, 30 July and after various periods of incubation.

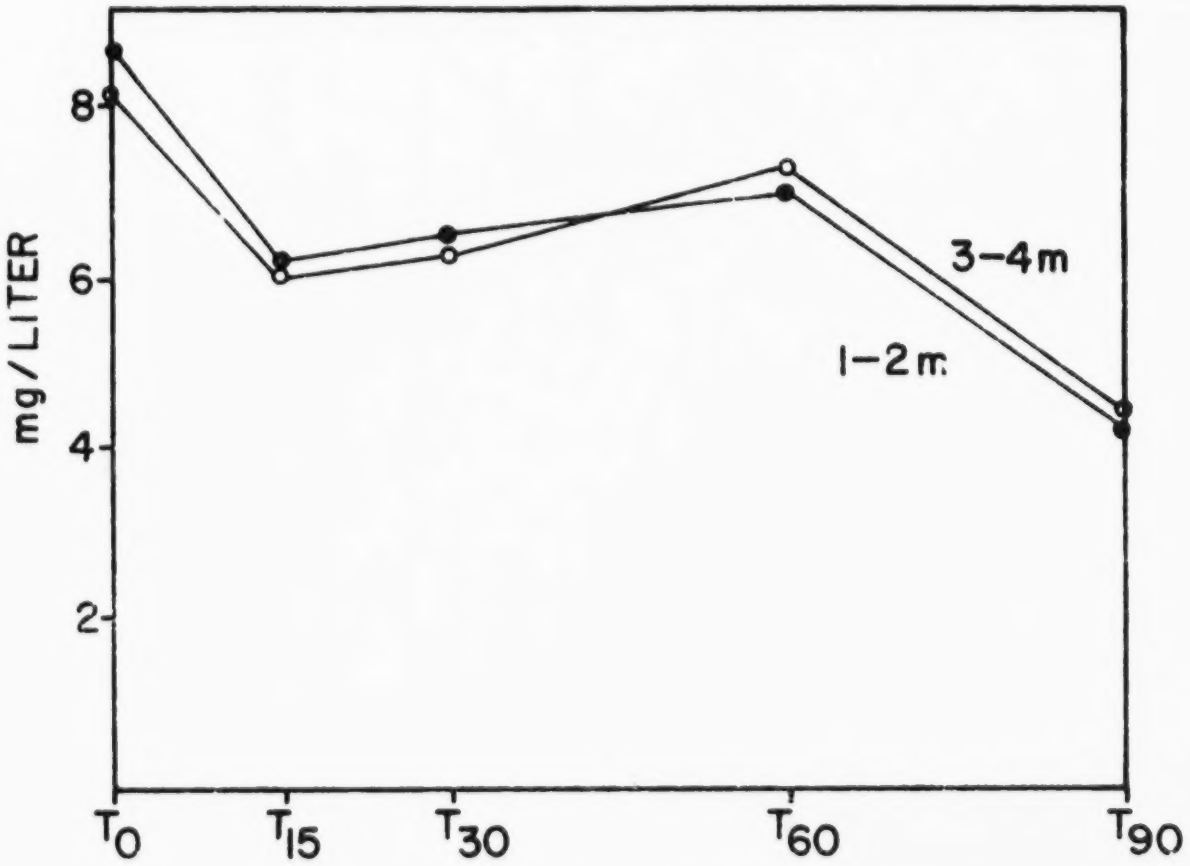


Figure 4. Vertical distribution of dissolved organic carbon in Cub Lake, 30 July and after various periods of incubation.

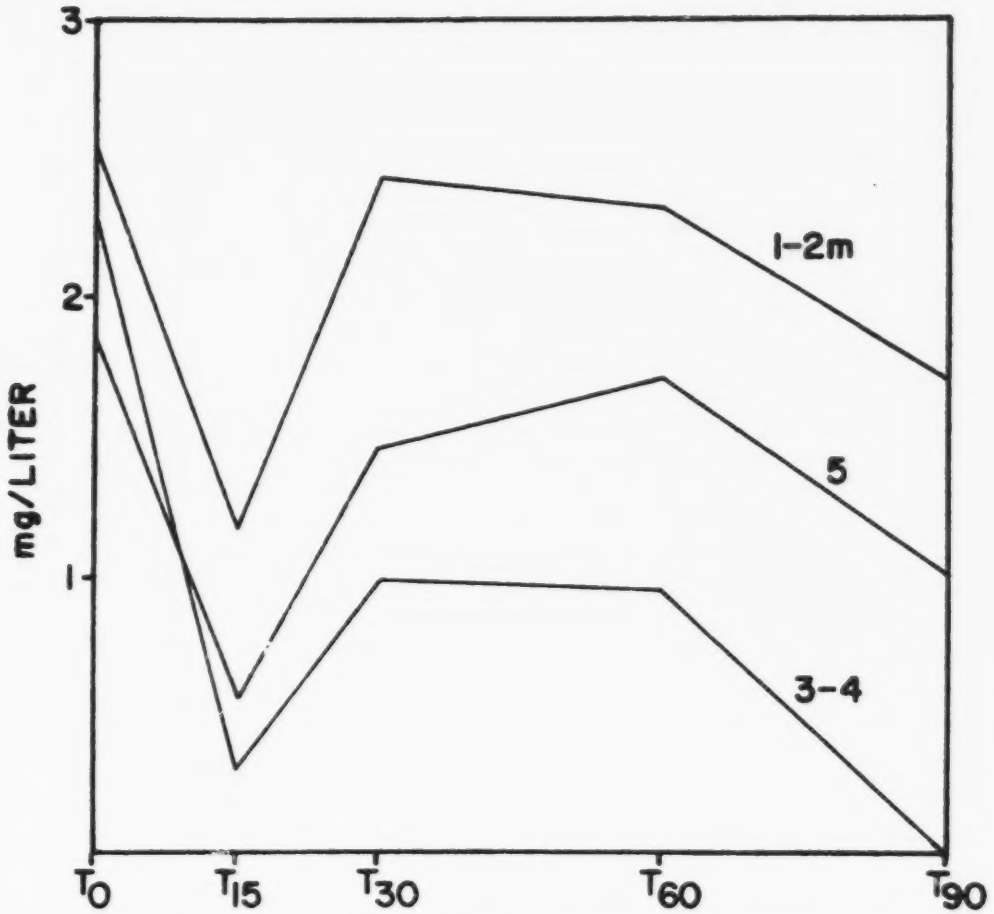


Figure 5. Vertical distribution of dissolved organic carbon in Green Lake, 11 August and after various periods of incubation.

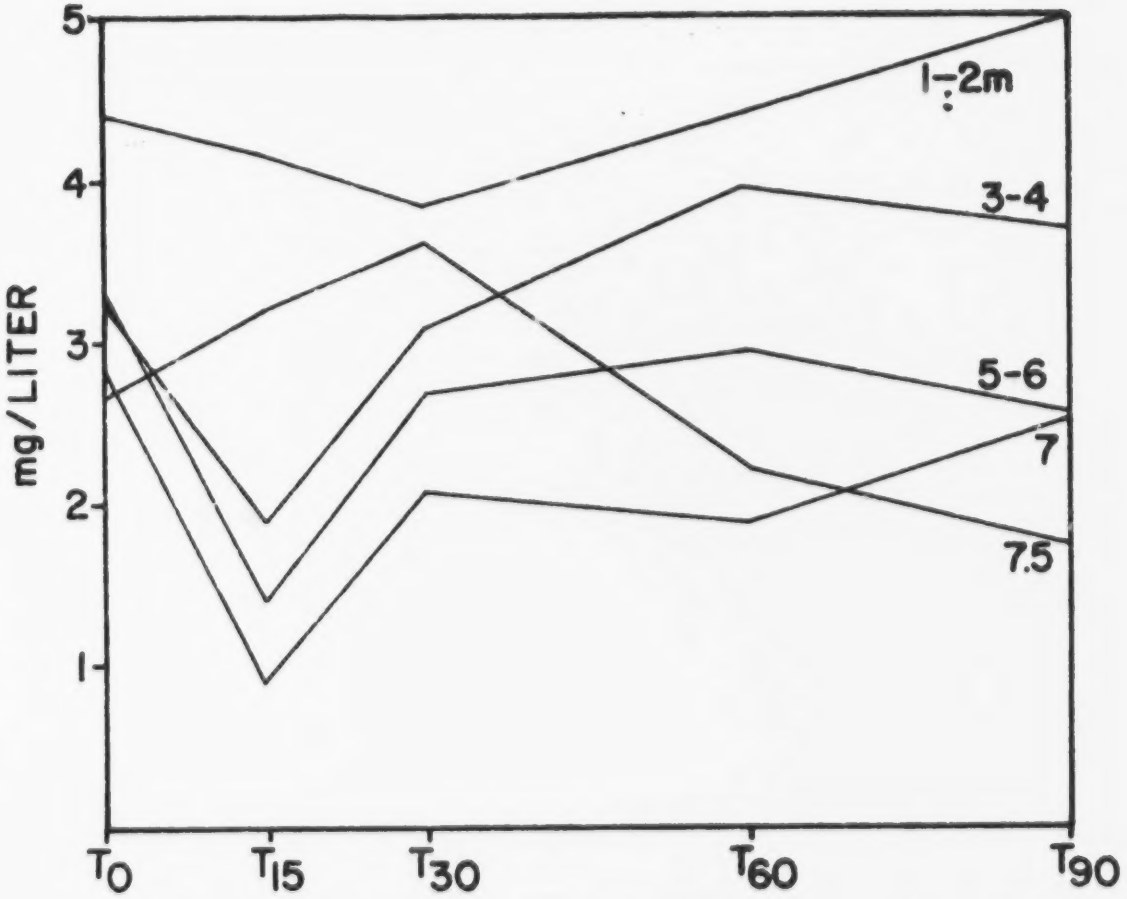


Figure 6. Vertical distribution of dissolved organic carbon in Bear Lake, 11 August and after various periods of incubation.

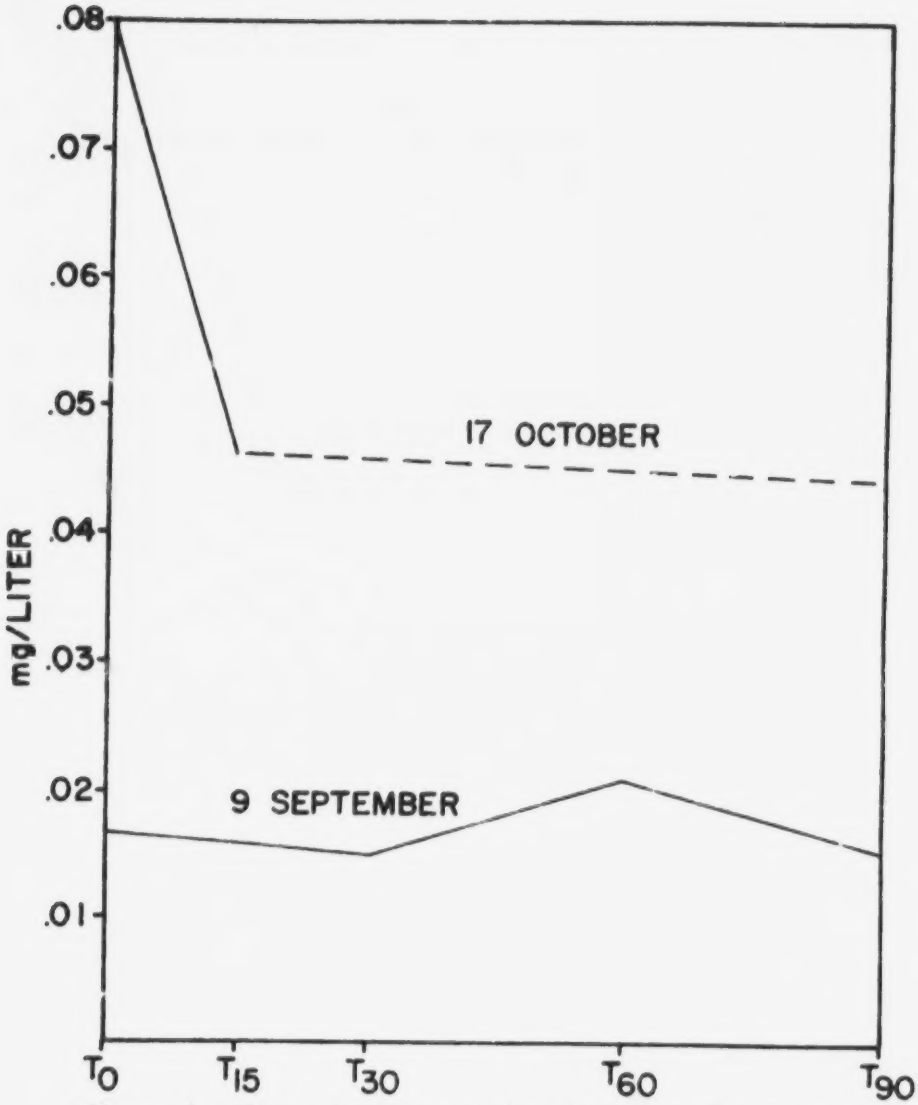


Figure 7. Mean net seston carbon in water column, Station A, Horsetooth Reservoir on two dates and after incubation.

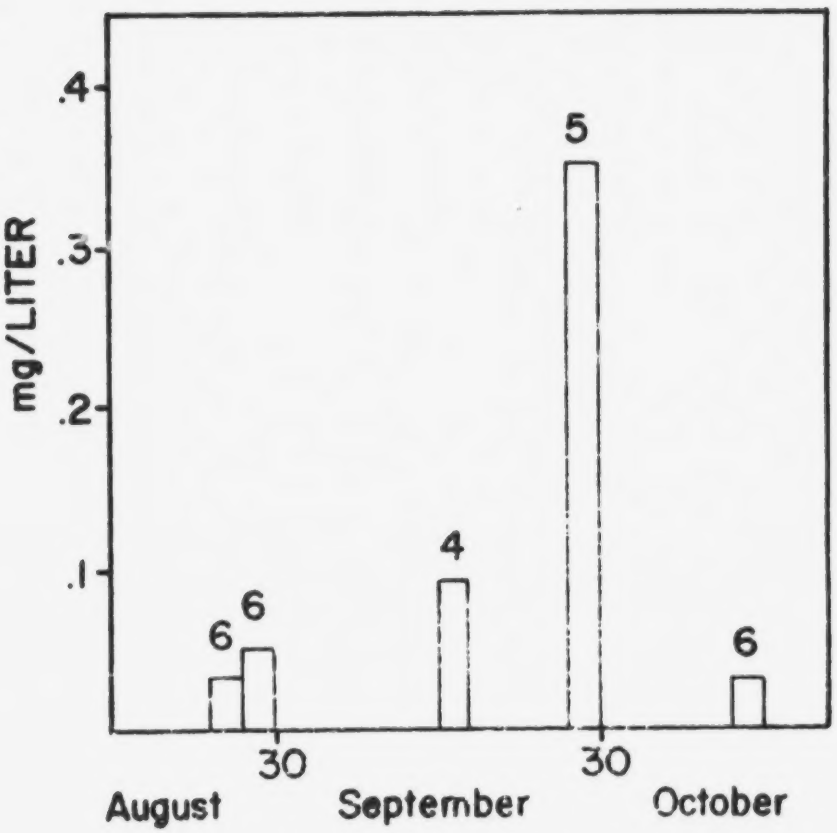


Figure 8. Mean net seston carbon at 1 m depth, Dixon Reservoir, five dates. Numbers above bars indicate number of replicate samples.

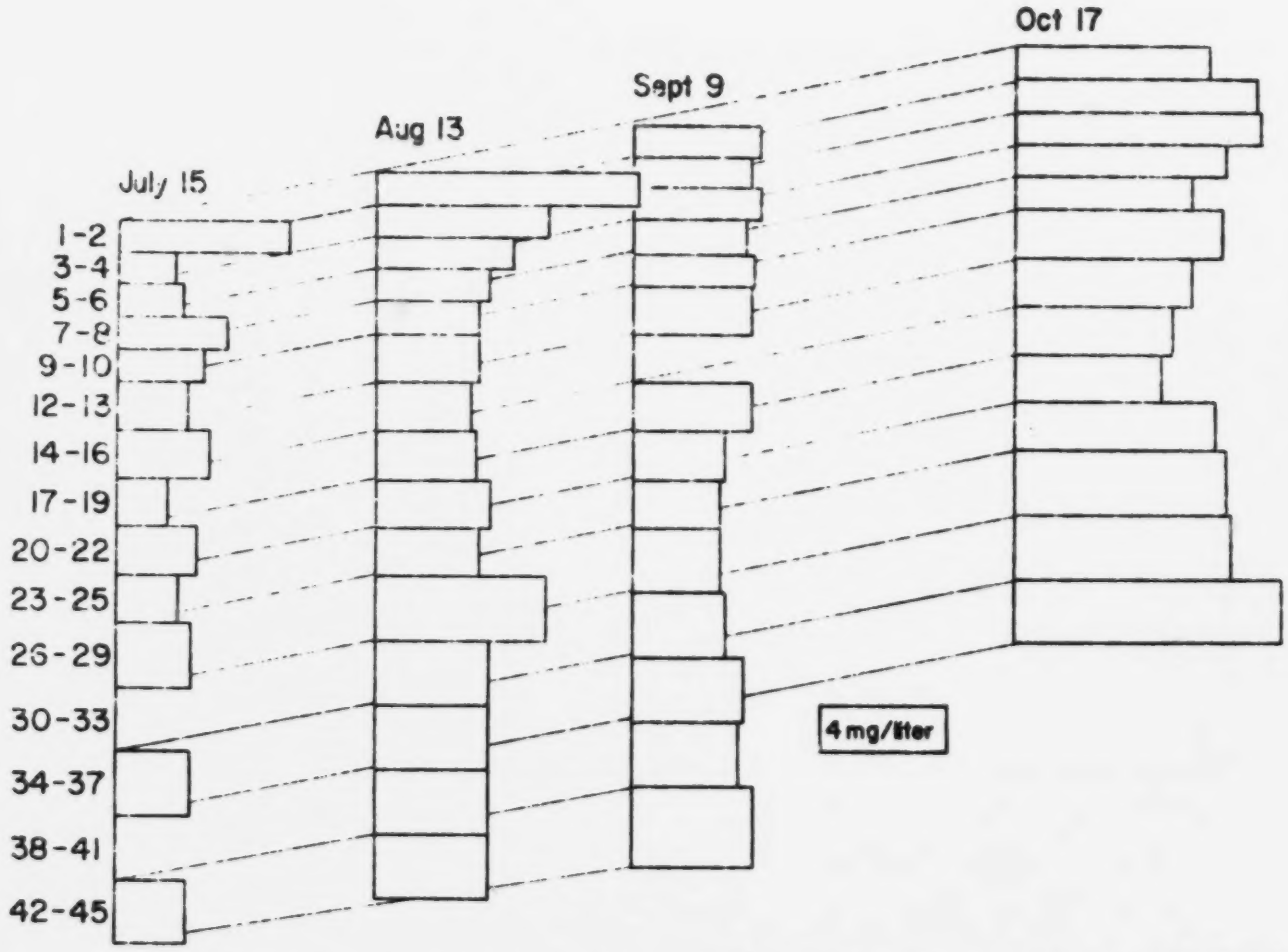


Figure 9. Vertical distribution of dissolved organic carbon, Station A, Horsetooth Reservoir.

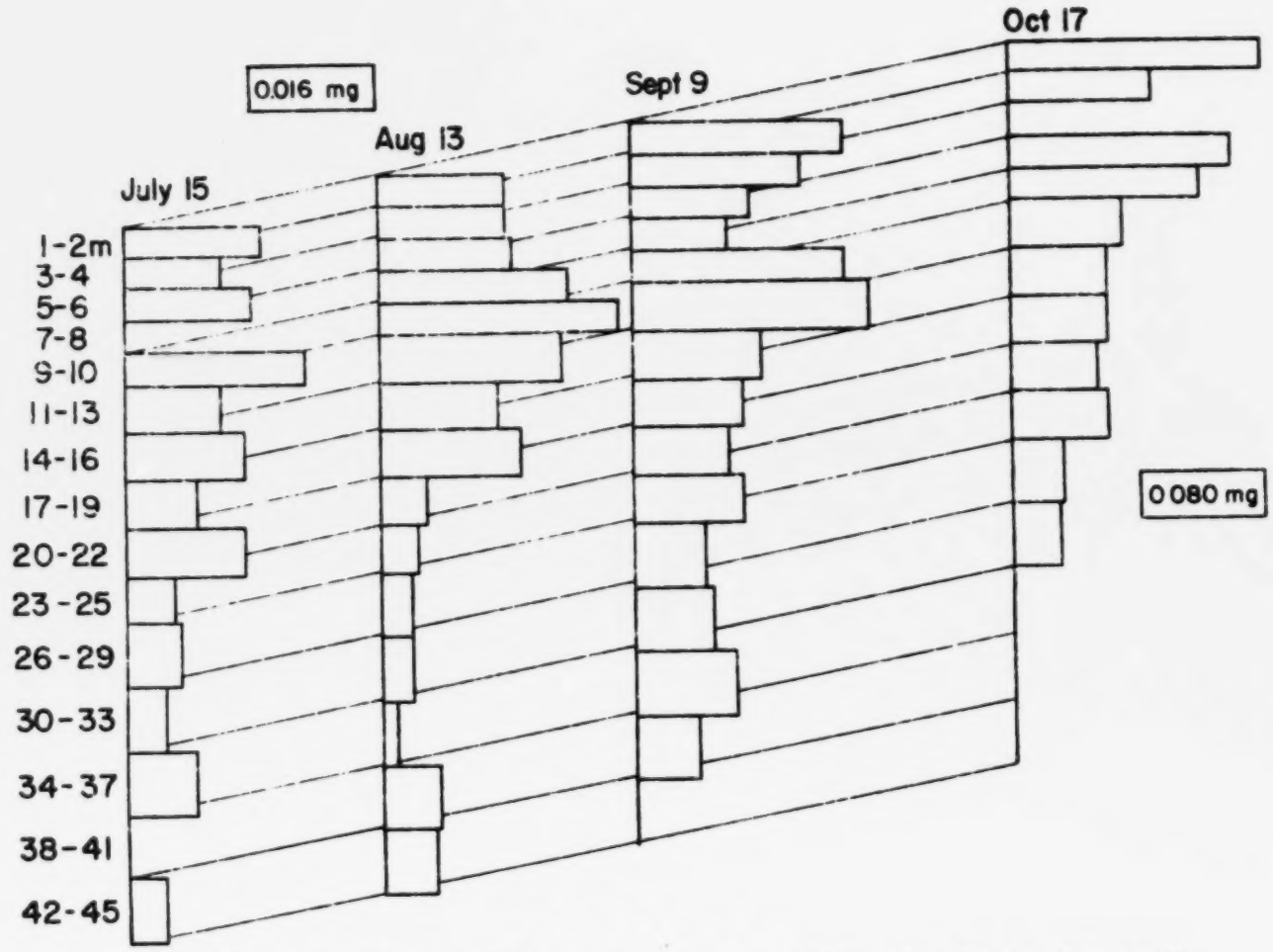


Figure 10. Vertical distribution of net seston carbon, Station A, Horsetooth Reservoir. Note change in scale for October.

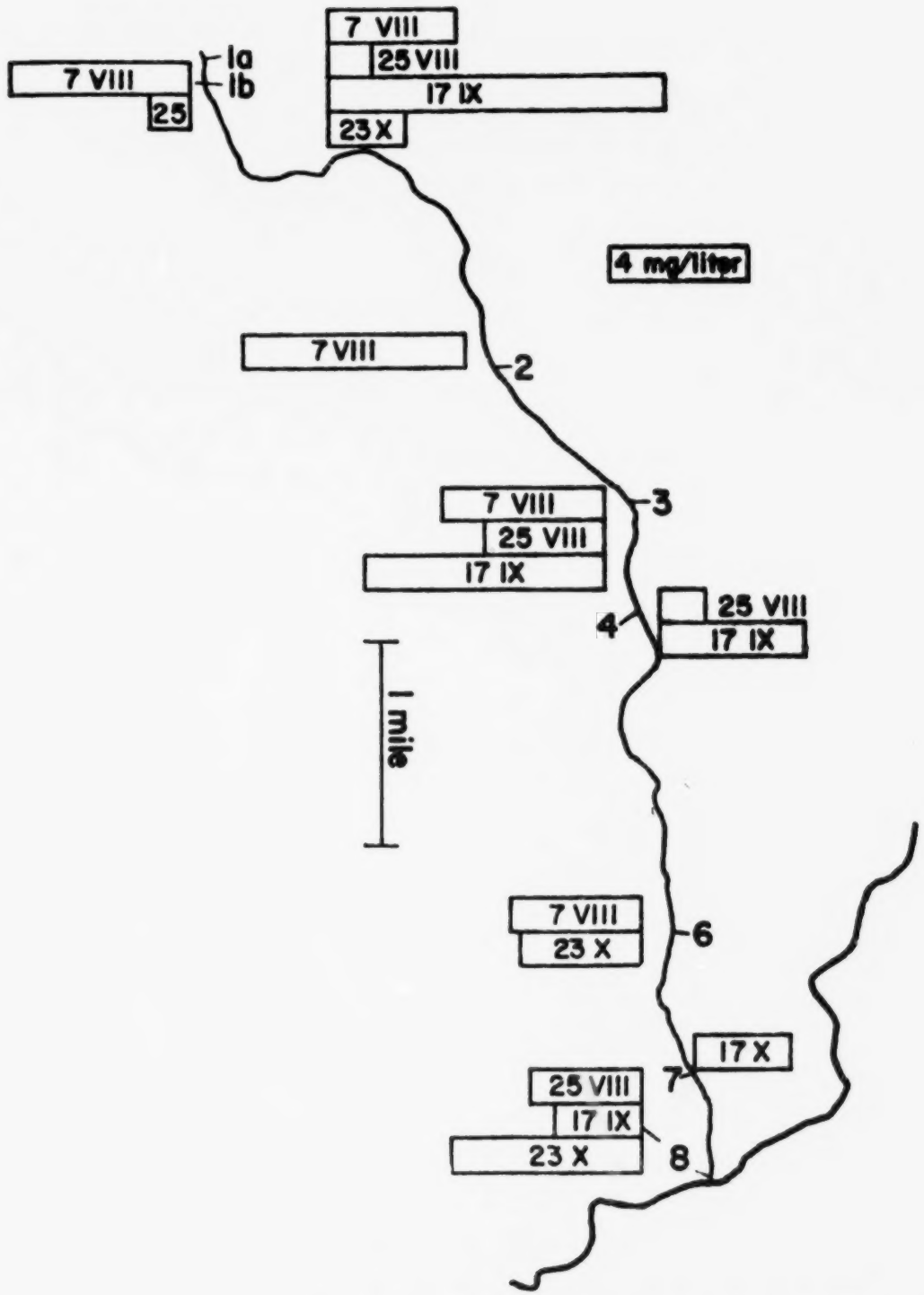


Figure 11. Temporal and spatial distribution of dissolved organic carbon in Spring Creek.

Table 11. Dissolved organic carbon (mg/liter) in Horsetooth Reservoir water (Station A) 15 July 1970 and after various periods of incubation.

| Sample depth, m | $T_o$ | $T_{15}$ | $T_{30}$ | $T_{60}$ | $T_{90}$ |
|---|---|---|---|---|---|
| 0 | - | - | - | - | - |
| 1-2 | 5.28 | 3.92 | 4.70 | 3.65 | 1.80 |
| 3-4 | 1.75 | 4.55 | 3.55 | 3.25 | 1.85 |
| 5-6 | 1.90 | 2.25 | 3.55 | 2.40 | 2.05 |
| 7-8 | 3.35 | - | 3.52 | 2.55 | 1.80 |
| 9-10 | 2.65 | 1.62 | 3.58 | 2.35 | 2.05 |
| 11-13 | 2.18 | - | 3.62 | 2.55 | 1.65 |
| 14-16 | 2.85 | - | 3.85 | 2.50 | 2.45 |
| 17-19 | 1.50 | 3.70 | 3.25 | 2.50 | 1.85 |
| 20-22 | 2.40 | 4.20 | 3.25 | 2.20 | 2.10 |
| 23-25 | 1.80 | 3.80 | 3.30 | 2.55 | 1.90 |
| 26-29 | 2.25 | 2.60 | 3.20 | 2.25 | 1.65 |
| 30-33 | - | 4.50 | 3.55 | 2.20 | 1.25 |
| 34-37 | 2.20 | 3.70 | 4.00 | 2.55 | 1.40 |
| 38-41 | - | 1.08 | 2.85 | 2.35 | 1.85 |
| 42-45 | 2.10 | - | 3.85 | 2.55 | 1.15 |
| $\bar{Y}$ | 2.48 | 3.26 | 3.57 | 2.56 | 1.79 |
| $100\ T_i/T_o$ | 100 | 131 | 144 | 103 | 72 |

Table 12. Dissolved organic carbon (mg/liter) in Horsetooth Reservoir water (Station A) 13 August 1970 and after various periods of incubation

| Sample depth, m | $T_o$ | $T_{15}$ | $T_{30}$ | $T_{60}$ | $T_{90}$ |
|---|---|---|---|---|---|
| 0 | 4.10 | | 4.05 | 1.30 | 2.25 |
| 1-2 | 8.20 | | 5.82 | 2.70 | 2.20 |
| 3-4 | 5.30 | | 6.60 | 3.75 | 4.40 |
| 5-6 | 4.25 | | 6.05 | 2.75 | 4.25 |
| 7-8 | 3.55 | | 6.95 | 3.25 | 3.00 |
| 9-10 | 3.35 | | 4.52 | 2.75 | 3.30 |
| 11-13 | 3.32 | | 3.67 | 3.30 | 3.25 |
| 14-16 | 3.05 | | 4.22 | 3.50 | 4.35 |
| 17-19 | 3.15 | | 4.30 | 2.05 | 2.55 |
| 20-22 | 3.50 | | 2.88 | 1.85 | 2.30 |
| 23-25 | 3.10 | | 3.07 | 2.45 | 3.30 |
| 26-29 | 5.38 | | 3.27 | 1.85 | 2.65 |
| 30-33 | 3.48 | | 3.32 | 1.60 | 2.25 |
| 34-37 | 3.50 | | 3.50 | 1.85 | 3.15 |
| 38-41 | 3.52 | | 3.18 | 1.95 | 2.35 |
| 42-45 | 3.52 | | 3.80 | 1.15 | 2.02 |
| $\bar{Y}$ | 4.02 | | 4.32 | 2.38 | 2.97 |
| 100 $T_i/T_o$ | 100 | | 107 | 59 | 74 |

Table 13. Dissolved organic carbon (mg/liter) in Horsetooth Reservoir water (Station A) 9 September 1970 and after various periods of incubation

| Sample depth, m | $T_o$ | $T_{15}$ | $T_{30}$ | $T_{60}$ | $T_{90}$ |
|---|---|---|---|---|---|
| 0 | 3.85 | 2.15 | 3.00 | | 3.60 |
| 1-2 | 3.95 | 2.50 | 2.40 | | 1.95 |
| 3-4 | 3.75 | 2.45 | 2.25 | | 2.05 |
| 5-6 | 3.95 | 2.40 | 2.65 | | 2.35 |
| 7-8 | 3.55 | 2.25 | 2.60 | | 2.10 |
| 9-10 | 3.80 | 2.30 | 2.40 | | 2.10 |
| 11-13 | 3.65 | 2.15 | 2.50 | | 2.10 |
| 14-16 | - | 2.05 | 3.00 | | 1.85 |
| 17-19 | 3.85 | 2.05 | 2.95 | | 1.70 |
| 20-22 | 2.90 | 2.05 | 2.85 | | 2.00 |
| 23-25 | 2.80 | 1.95 | 2.50 | | 2.50 |
| 26-29 | 2.80 | 2.00 | 2.30 | | 2.70 |
| 30-33 | 2.90 | 1.85 | 2.55 | | 2.25 |
| 34-37 | 3.40 | 1.70 | 2.90 | | 2.90 |
| 38-41 | 3.20 | 1.95 | 2.40 | | 2.65 |
| 41-45 | 3.85 | 2.15 | 3.00 | | - |
| $\bar{Y}$ | 3.48 | 2.12 | 2.64 | | 2.32 |
| $100\ T_j/T_o$ | 100 | 62 | 76 | | 67 |

Table 14. Dissolved organic carbon (mg/liter) in Horsetooth Reservoir water (Station A) 17 October 1970 and after various periods of incubation

| Sample depth, m | $T_o$ | $T_{15}$ | $T_{30}$ | $T_{60}$ | $T_{90}$* |
|---|---|---|---|---|---|
| 0 | 5.85 | 4.05 | | | - |
| 1-2 | 5.95 | 3.50 | | | 5.25 |
| 3-4 | 7.50 | 4.15 | | | - |
| 5-6 | 7.62 | 5.10 | | | 6.87 |
| 7-8 | 6.65 | 4.40 | | | 4.60 |
| 9-10 | 5.42 | 4.90 | | | 5.75 |
| 11-13 | 6.38 | 3.90 | | | 5.60 |
| 14-16 | 5.50 | 4.25 | | | 5.15 |
| 17-19 | 4.82 | 2.20 | | | 5.75 |
| 20-22 | 4.52 | 3.00 | | | 3.62 |
| 23-25 | 6.22 | 2.80 | | | 3.90 |
| 26-29 | 6.55 | 2.85 | | | 5.50 |
| 30-33 | 6.68 | 2.95 | | | 3.82 |
| 34-37 | 8.35 | 3.00 | | | 5.95 |
| $\bar{Y}$ | 6.29 | 3.65 | | | 5.15 |
| 100 $T_i/T_o$ | 100 | 58 | | | 82 |

* average of three determinations/depth

Table 15. Dissolved organic carbon and net seston carbon in Horsetooth Reservoir water (Station C) 19 August 1970

| Sample depth, m | DOC mg/liter | NSC mg/liter |
|---|---|---|
| 0 | 2.5 | - |
| 1 | 3.6 | .043 |
| 2 | 3.6 | .034 |
| 3 | 3.3 | .041 |
| 4 | 3.3 | .035 |
| 5 | 3.6 | - |
| 6 | 3.4 | .041 |
| 7 | 3.4 | .044 |
| 8 | 3.2 | .043 |
| 9 | 3.4 | .042 |
| 10 | 4.0 | .044 |
| 11 | 2.8 | .043 |
| 12 | 2.0 | .029 |
| 13 | 2.2 | .031 |
| 14 | 2.4 | .025 |
| $\bar{Y}$ | | .038 |

Table 16. Dissolved organic carbon (mg/liter) in Bear Lake water 30 July and 23 September 1970 and after incubation

30 JULY

| Sample depth, m | $T_o$ | $T_{15}$ | $T_{30}$ | $T_{60}$ | $T_{90}$ |
|---|---|---|---|---|---|
| 1-2 | 5.43 | 3.85 | 3.35 | 4.55 | 2.55 |
| 3-4 | 4.97 | 3.72 | 3.02 | 3.95 | 2.15 |
| 5-6 | 4.85 | 3.62 | 3.10 | 4.00 | 2.30 |
| 7-8 | 5.42 | 3.72 | 3.15 | 4.25 | 2.50 |
| 9-10 | 5.98 | 3.60 | 3.45 | 4.95 | 2.85 |
| $\bar{Y}$ | 5.30 | 3.70 | 3.21 | 4.34 | 2.47 |
| 100 $T_i/T_o$ | 100 | 70 | 61 | 82 | 47 |

23 SEPTEMBER

| | | | |
|---|---|---|---|
| 1-2 | 5.00 | 3.90 | |
| 3-4 | 4.60 | 3.70 | Samples lost |
| 5-6 | 3.90 | 3.15 | |
| 7-8 | 4.25 | 3.75 | |
| $\bar{Y}$ | 4.44 | 3.62 | |
| 100 $T_i/T_o$ | 100 | 82 | |

Table 17. Dissolved organic carbon (mg/liter) in Cub Lake water 30 July 1970 and after incubation

| Sample depth, m | $T_o$ | $T_{15}$ | $T_{30}$ | $T_{60}$ | $T_{90}$ |
|---|---|---|---|---|---|
| 1-2 | 8.70 | 6.25 | 6.50 | 7.05 | 4.25 |
| 3-4 | 8.20 | 6.08 | 6.30 | 7.25 | 4.40 |
| $\bar{Y}$ | 8.45 | 6.16 | 6.40 | 7.15 | 4.32 |
| 100 $T_i/T_o$ | 100 | 73 | 76 | 85 | 51 |

Table 18. Dissolved organic carbon (mg/liter) in Green Lake water 11 August and 3 September 1970 and after incubation

11 AUGUST

| Sample depth, m | $T_o$ | $T_{15}$ | $T_{30}$ | $T_{60}$ | $T_{90}$ |
|---|---|---|---|---|---|
| 0 | 1.30 | - | - | - | - |
| 1-2 | 2.50 | 1.15 | 2.42 | 2.30 | 1.70 |
| 3-4 | 2.30 | 0.30 | 0.98 | 0.95 | 0.0 |
| 5 | 1.85 | 0.55 | 1.45 | 1.70 | 1.00 |
| $\bar{Y}$ | 2.22 | 0.67 | 1.62 | 1.65 | 0.90 |
| $100\ T_i/T_o$ | 100 | 30 | 73 | 74 | 40 |

3 SEPTEMBER

| | | |
|---|---|---|
| 1-2 | 2.32 | |
| 3-4 | 1.84 | Samples lost |
| 5-6 | 1.20 | |
| $\bar{Y}$ | 1.80 | |

Table 19. Dissolved organic carbon (mg/liter) in Blue Lake water 11 August and 3 September 1970 and after incubation

11 AUGUST

| Sample depth, m | $T_o$ | $T_{15}$ | $T_{30}$ | $T_{60}$ | $T_{90}$ |
|---|---|---|---|---|---|
| 0 | 4.60 | - | - | - | - |
| 1-2 | 4.40 | 4.35 | 3.85 | 4.40 | 5.00 |
| 3-4 | 3.25 | 1.90 | 3.12 | 3.95 | 3.70 |
| 5-6 | 3.30 | 1.40 | 2.70 | 2.95 | 2.55 |
| 7 | 2.85 | 0.90 | 2.12 | 1.90 | 2.50 |
| 7.5 | 2.65 | 3.20 | 3.60 | 2.20 | 1.75 |
| $\bar{Y}$ | 3.29 | 2.35 | 3.08 | 3.08 | 3.10 |
| 100 $T_i/T_o$ | 100 | 71 | 94 | 94 | 94 |

3 SEPTEMBER

| | | |
|---|---|---|
| 1-2 | 3.60 | |
| 3-4 | 3.32 | Samples lost |
| 5-6 | 1.42 | |
| 7-8 | 2.87 | |
| $\bar{Y}$ | 2.80 | |

Table 20. Net seston carbon (mg/liter) Horsetooth Reservoir (Station A) two dates 1970

| | Station A 15 July | | 13 August |
|---|---|---|---|
| Sample depth, m | $T_o$ | $T_{60}$ | $T_o$ |
| 1-2 | .0176 | .033 | .0165 |
| 3-4 | .0125 | - | .0167 |
| 5-6 | .0158 | - | .0171 |
| 7-8 | - | .039 | .0237 |
| 9-10 | .0225 | - | .0303 |
| 11-13 | .0125 | .018 | .0233 |
| 14-16 | .0159 | .021 | .0154 |
| 17-19 | .0093 | - | .0178 |
| 20-22 | .0149 | - | .0064 |
| 23-25 | .0060 | .018 | .0048 |
| 26-29 | .0073 | - | .0039 |
| 30-33 | .0047 | .015 | .0039 |
| 34-37 | .0087 | - | .0020 |
| 38-41 | - | .012 | .0069 |
| 42-45 | .0049 | .016 | .0069 |
| $\overline{Y}$ | .0117 | .022 | .0130 |
| $100\ T_i/T_o$ | 100 | 188 | |

Table 21. Net seston carbon (mg/liter) Horsetooth Reservoir (Station A) 9 September 1970 and after incubation

| Sample depth, m | $T_o$ | $T_{15}$ | $T_{30}$ | $T_{60}$ | $T_{90}$ |
|---|---|---|---|---|---|
| 1-2 | .0270 | | .0208 | .034 | .019 |
| 3-4 | .0216 | | .0212 | .034 | .019 |
| 5-6 | .0154 | | .0207 | .026 | .032 |
| 7-8 | .0120 | | .0129 | .029 | - |
| 9-10 | .0272 | | .0185 | .024 | .014 |
| 11-13 | .0297 | | .0244 | .025 | .017 |
| 14-16 | .0165 | | .0174 | .030 | .042 |
| 17-19 | .0140 | | .0134 | .044 | .019 |
| 20-22 | .0120 | | .0132 | .025 | .014 |
| 23-25 | .0137 | | .0118 | .023 | - |
| 26-29 | .0090 | | .0065 | .015 | .001 |
| 30-33 | .0097 | | .0064 | .002 | .002 |
| 34-37 | .0130 | | .0105 | .013 | .008 |
| 38-41 | .0082 | | .0032 | .041 | .011 |
| $\bar{Y}$ | .0164 | | .0144 | .026 | .015 |
| $100\ T_i/T_o$ | 100 | | 88 | 162 | 94 |

Table 22. Net seston carbon (mg/liter) Horsetooth Reservoir (Station A) 17 October 1970 and after incubation

| Sample depth, m | $T_o$ | $T_{15}$ | $T_{30}$ | $T_{60}$ | $T_{90}$* |
|---|---|---|---|---|---|
| 1-2 | .157 | .096 | | | .0731 |
| 3-4 | .091 | .067 | | | .0491 |
| 5-6 | - | .074 | | | .0633 |
| 7-8 | .141 | .062 | | | .0729 |
| 9-10 | .118 | .045 | | | .0446 |
| 11-13 | .068 | .033 | | | .0436 |
| 14-16 | .059 | .029 | | | .0375 |
| 17-19 | .062 | .040 | | | .0380 |
| 20-22 | .055 | .040 | | | .0402 |
| 23-25 | .062 | .034 | | | .0367 |
| 26-29 | .035 | .016 | | | .0200 |
| 30-33 | .029 | .015 | | | .0219 |
| 34-37 | - | - | | | .0263 |
| $\overline{Y}$ | .080 | .046 | | | .0436 |
| 100 $T_i/T_o$ | 100 | 58 | | | 55 |

Table 23. Net seston carbon (mg/liter) in three lakes

BEAR LAKE - 30 JULY 1970

| Sample depth, m | $T_o$ | $T_{15}$ | $T_{30}$ | $T_{60}$ | $T_{90}$ |
|---|---|---|---|---|---|
| 1-2 | .0764 | - | .0050 | - | - |
| 3-4 | .0461 | - | .0059 | - | - |
| 5-6 | .0456 | - | .0046 | - | - |
| 7-8 | .0643 | - | .0052 | - | - |
| 9-10 | .0415 | - | .0045 | - | - |
| $\bar{Y}$ | .0578 | | .0050 | | |
| 100 $T_i/T_o$ | 100 | | 9 | | |

BEAR LAKE - 23 SEPTEMBER

| | | | | | |
|---|---|---|---|---|---|
| 1-2 | .0066 | - | - | - | - |
| 3-4 | .0167 | - | - | - | - |
| 5-6 | .0098 | - | - | - | - |
| 7-8 | .0186 | - | - | - | - |

BLUE LAKE - 3 SEPTEMBER

| | | | | | |
|---|---|---|---|---|---|
| 1-2 | .045 | - | - | .0039 | - |
| 3-4 | .045 | .046 | - | .0025 | - |
| 5-6 | .048 | .051 | - | .0039 | - |
| 7 | .059 | - | - | .0031 | - |
| $\bar{Y}$ | .049 | .048 | | .0034 | |
| 100 $T_i/T_o$ | 100 | | | 7 | |

GREEN LAKE - 3 SEPTEMBER

| | | | | | |
|---|---|---|---|---|---|
| 1-2 | .0261 | .021 | - | - | - |
| 3-4 | .0228 | .011 | - | .0023 | - |
| 5-6 | .0393 | .051 | - | .0023 | - |
| $\bar{Y}$ | .0294 | .028 | | .0023 | |
| 100 $T_i/T_o$ | 100 | | | 9 | |

Table 24. Dissolved organic carbon, net seston carbon and dry weight of net seston, Bear Lake 23 September 1970

| Sample depth, m | DOC* mg/liter | NSC** mg/liter | dry weight** mg/liter | 100c/ dry wt. |
|---|---|---|---|---|
| 1-2 | 5.00 | .0066 | .26 | 2.5 |
| 3-4 | 4.60 | .0167 | .41 | 4.1 |
| 5-6 | 3.90 | .0098 | .12 | 8.2 |
| 7-8 | 4.25 | .0186 | .40 | 4.6 |

* mean of two determinations/depth

** " " three " "

Table 25. Carbon content and dry weight of net seston, Dixon Reservoir. All samples from 1 meter depth

| | 29 SEPTEMBER | |
|---|---|---|
| | dry weight mg/liter | |
| n | 6 | 5 |
| $\bar{Y}$ | 2.23 | .3520 mg/liter |
| $s^2$ | .3143 | .0303 |
| s | .561 | .174 |
| SE | .229 | .078 |
| 95% limits | 1.64-2.82 | .1360-.4880 |
| error | 10 | 22 |
| CV | 25 | 50 |

| | 12 OCTOBER | |
|---|---|---|
| n | 8 | 6 |
| $\bar{Y}$ | 4.05 | 96.33 % absorbance* |
| $s^2$ | .1914 | 1.26 |
| s | .437 | 1.123 |
| SE | .1545 | .4586 |
| 95% limits | 3.69-4.41 | 95.15-97.51 |
| error | 4 | .5 |
| CV | 10 | 1 |

* .0293 mg carbon

Table 26. Carbon content and dry weight of net seston, Horsetooth Reservoir (Station A) 17 October 1970

| Sample depth, m | dry weight mg/liter | carbon mg/liter |
|---|---|---|
| 1-2 | 1.20 | .157 |
| 3-4 | 1.10 | .091 |
| 5-6 | 1.20 | - |
| 7-8 | 1.60 | .141 |
| 9-10 | 1.20 | .118 |
| 11-13 | 0.80 | .068 |
| 14-16 | 0.80 | .059 |
| 17-19 | 0.67 | .062 |
| 20-22 | 0.53 | .055 |
| 23-25 | 0.60 | .062 |
| 26-29 | 0.35 | .035 |
| 30-33 | 0.05 | .029 |
| 34-37 | 0.30 | - |
| $\bar{Y}$ | 0.80 | .080 |

## SECTION VI

## FIELD AND LABORATORY RESULTS AFTER 1 JANUARY 1971

Introduction - In view of fluctuations in carbon concentrations both in natural waters and in samples incubated in the laboratory, it was deemed desirable to narrow the sampling scope and to increase frequency of sampling. Field work was concentrated on, but not confined to, two small bodies of water, Bear Lake and Dixon Reservoir (Table 27) and on two small streams.

Bear Lake was sampled at approximately weekly intervals. Water was taken from each meter of depth from the ice surface to maximum depth. Net seston was strained from 500 mls of water and variable but known volumes, usually 100 to 300 mls., of strained water were filtered for filter seston carbon. DOC was determined from 5 ml samples.

Dixon Reservoir was sampled at intervals ranging from 1 to 7 days. All samples were taken at a depth of 1 m.; net seston, filter and dissolved carbon values were determined.

Two small streams, one, Spring Creek, had already received some sampling prior to 1 January. Glacier Creek rises in tarns and snowfields at high altitudes in Rocky Mountain National Park and in addition to other sources received the small outflow from Bear Lake. Glacier Creek was sampled at four stations, ranging in altitude from 9220 feet down to 8000.

Laboratory procedures remained as before except that a filter fraction was added. This was made possible by acquisition of an electric oven that could be controlled so that glass fiber filters could be preheated just below the melting point. Filters were preheated for 2 hours.

Experiments using blank, preheated filters revealed that they caused absorbency when treated and processed through the infrared analyzer; however, dispersion among replicates was small. Thus, blank filters were run with each batch of filters containing seston. An average percentage of absorbency for blanks was figured and this amount subtracted from the percentage of absorbency of each seston filter prior to conversion to carbon value.

Three incubation experiments were conducted. In the first, a 10-gallon polyethylene carboy was filled with water and included seston from Dixon Reservoir. In the laboratory, the carboy was covered by a large cardboard box to exclude light. The carboy was sampled for NSC, FSC, and DOC over a three-week period. Adequate stirring of the carboy prior to sub-sampling created problems, thus another incubation series was run.

This time a number of 1-liter polyethylene bottles were filled with Dixon Reservoir water and seston. These were stored in the dark in the laboratory and sampled over a three-week period. Each bottle could be

thoroughly mixed prior to sub-sampling and the contents of each were used completely. Despite our precautions, it is probable that the bottles received slightly unequal amounts of seston during filling at the Reservoir.

Because the presence of live large zooplankters could have affected the previous incubation experiments, a new one was initiated on 29 April.

Using a Kemmerer water bottle, 30 l of water was obtained from a depth of 1 m in Dixon Reservoir. In the laboratory, the container filled with lake water was submerged in a water bath at 80C and held there for 30 minutes after the lake water had reached 80C. This was done to kill crustaceans and other large zooplankters.

After heating, the lake water was thoroughly mixed and poured into a number of one liter polyethylene bottles and allowed to cool to room temperature; approximately 2-3 mls of raw unheated lake water were added to provide bacterial seeding.

The bottles were tightly capped and stored in a dark box at room temperature.

On nine occasions between 29 April and 24 May, two bottles were removed from the dark box and their contents analyzed for dissolved, filter and net fractions of organic carbon. Each bottle was handled separately, thus giving duplicate samples.

Bear Lake - DOC ranged from about 2.5 to 10 mg/liter over nine sampling dates (Figure 12). Most values were between 3.5 and 5.5 mg C/liter, however. Values for 23 January are so much greater than on most other dates that error is suggested. DOC tended to be concentrated in the deeper waters. No overall increase or decrease with time is immediately evident in Figure 12.

NSC in Bear Lake did not exhibit clear-cut trends in changeover time nor were there consistent patterns of depth distribution (Figure 13), although the maximum value for any sampling tended not to be found in the deepest water. Noticeable fractions of the net seston were the copepod Diaptomus nudus and the cladoceran Daphnia rosa. These animals are able to adjust their positions in the water column in response to a number of changing environmental factors including light intensity. Vertical migrations would tend to confound distributional patterns in net seston.

In contrast to NSC, FSC tended to accumulate in deep water, just above the lake bottom (Figure 14). Observations of previous years (Reed 1970 and unpublished) have revealed the accumulation of autochthonous detritus in the deep water of Bear Lake during winter stagnation and summer stratification. Ratios of NSC : FSC : DOC were approximately .02 : .20 : 1.0; i.e. DOC was 50 times greater than NSC, but only 5 times FSC.

During the period of these observations, Bear Lake was covered by 65-80 cm of ice; snow depths ranged from 0 to 75 cm.

Dixon Reservoir - Between 9 February and 24 March, DOC varied from 5.82 to 10.70 mg/liter and averaged 6.71. FSC averaged .4933 mg/liter and ranged from .2900 to .9438 mg. The range and mean for NSC were .0487 to .2250 and .1187 mg/liter. Mean DOC value was 13 times that of FSC and 60 times greater than mean NSC; FSC was about 5 times larger than NSC. All fractions fluctuated greatly and without clear patterns (Figure 15). Without direct microscopic examination, one cannot say whether an increase in particulate fraction was the result of synthetic or destructive processes; i.e. a pulse of small plankters could swell FSC values as well as detrital particles resulting from die-off and decay of a plankter population. Moreover, the relationships among NSC, FSC and DOC are more likely to result from some past condition rather than to be the result of conditions obtaining at the moment of sampling. On two occasions, decrease in DOC was contemporary with increase in particulate carbon. Forsberg (1967) attributed simultaneous decrease in dissolved carbon with increased chlorophyll to uptake by phytoplankters.

Spring Creek - DOC in Spring Creek ranged from about 2 to 8 mg/liter (Figure 16). Spring Creek winds along the edge of Fort Collins and receives agricultural and suburban drainage, in addition to roadside ditches. Correlation of changes in DOC with respect to precipitation has not yet been examined; perhaps peaks may have resulted from inputs of allochthonous carbon. In any event, the sampling stations did not retain relative rankings, although stations 4 and 6, those furthermost downstream, often had greatest carbon values.

Glacier Creek - DOC in Glacier Creek also fluctuated between stations and over time (Figure 17), although not as widely as in Spring Creek. On 29 March, stations 3 and 4 were obviously being influenced by local snowmelt and runoff. At higher elevations, the snow was not yet melting. Glacier Creek in winter is smaller than Spring Creek and certainly seems more thoroughly isolated from its surroundings by ice and snow than does Spring Creek.

Incubation Studies - Results of the first incubation run, Dixon carboy, are shown in Figure 18. DOC increased as FSC decreased and then declined irregularly to about the initial value. After an increase following about a week of incubation, FSC remained below initial value, although fluctuating. NSC exhibited an enormous increase after a week of incubation; this value is so extreme relative to others that the possibility of error is very difficult to dismiss. Live Daphnia and copepods were observed in the carboy at the close of the experiment, thus the one high value of NSC could have resulted from a chance concentration of large zooplankters in the subsamples. There is no clear indication that amount of organic carbon in the carboy decreased appreciably during incubation. Possible destruction of carbon may have been offset by

heterotrophic $CO_2$ fixation and it is conceivable that sufficient light reached the carboy to permit some photosynthesis, although it would surely have been carried on at a very low light level.

The last incubation series, Dixon bottle, (Figure 19) is no less bewildering than Dixon carboy. Again, there is no indication that there was less total carbon at the conclusion of incubation than at the start; in fact, NSC and FSC appear to have increased. In this experiment, photosynthesis can be ruled out; the bottles were stored in complete darkness. Live zooplankters were, however, still present in the final bottle analyzed.

A number of unknowns come to mind: carbon fixation by heterotrophic bacteria and facultative heterotrophic algae, "surface effect" which would be largely relative to the volume of each liter bottle. If bacteria grew profusely on the inner surfaces of the bottles, they could be dislodged in mixing intended to suspend net seston and thus perhaps appear in FSC. Dynamics of zooplankton metabolism probably also affected different carbon fractions in unknown ways.

The results of the third incubation experiment are summarized in Table 28. Prior to heating on 29 April, Dixon Reservoir water contained a total of nearly 7 mg organic carbon/liter, of which 6.19 was DOC, .042 NSC, and .758 FSC.

Concentrations of fractions exhibited a decrease between 29 April and 3 May (Table 28). All increased in the next two days and all decreased between 17 May and 19 May, otherwise, there were no common trends of increase or decrease. Between 14 and 17 May, DOC and NSC increased substantially, but FSC decreased slightly.

After the period of initial decrease and recovery, i.e. 10 May through 24, DOC fluctuated little, about a mean value of 5.25 mg/l. NSC was more variable, about a mean concentration of .036 mg/l, but no clear trends are evident. Certainly the data do not suggest a progressive loss of carbon from large particles. FSC varied about a mean value of .609 mg/l and, as was the case with NSC, did not exhibit a clear trend toward diminution as incubation proceeded.

The data are few and possible uneven distribution of particulate carbon among the liter bottles and problems of analysis of filter seston add to the difficulties of drawing firm conclusions from this experiment. If the bottles can be regarded as closed systems, i.e. the caps were tight enough to permit no passage of gaseous carbon dioxide and no exchanges of carbon between bottle walls and contents occurred. The organic carbon behaved in a very conservative fashion. The initial amount of organic carbon was 6.99 mg/l., assuming the final value was 5.94; then about 1 mg of organic carbon was lost per liter of water. The rate of loss was about 0.04 mg/l day.

Considering only the first post-heating sample, about 2 mg organic carbon were lost per liter of reservoir water; most of which was recovered by the time of the second post-heating sampling. If the period between heating and 5 May is regarded as a time of readjustment and stabilization, one may compare the total organic carbon present on that date (6.66) with that at the conclusion of the experiment (5.94) to arrive at a loss of 0.7 mg/l and .037 mg/l, day.

The presence of large, live zooplankters seems not to have confounded this experiment as it might have in earlier incubation attempts. Warming to 80C was lethal to cladocerans and copepods in the water. Possibly eggs, nauplii or other immature forms could have been introduced to some bottles at the time raw, unheated Dixon Reservoir water was added; no live cladocerans or copepods were observed, however. Live zooplankters are not regarded as having influenced the outcome of this experiment in any way.

Possible roles of viable small zooplankters are not so easy to assess. Conceivably some rotifers or protozoans might have survived the heating and certainly could have been introduced unnoticed with the raw water. Bacteria are, of course, the most significant unknown.

A major assumption of this procedure was that bacteria would either survive the heating or be introduced into the bottles in the unheated water. Should either failed to have happened and the bottles resulted in a series of sterile environments, changes in carbon could be regarded as fluctuations due to error in measuring a constant amount of carbon - a possible but highly unlikely situation.

Regarding each bottle as a closed system, it may conceptually be diagrammed as having two carbon components, one inorganic and one organic.

| Inorganic carbon | Organic carbon |
|---|---|
| carbon dioxide | dissolved |
| bicarbonate | |
| carbonate | particulate |

Within each box there may be exchanges between forms and states, e.g. particulate carbon may become dissolved, or bicarbonate give up $CO_2$, etc. There are, of course, exchanges between boxes. In the case of this experiment, there appears to have been a net loss from the organic carbon component to presumably the inorganic box. In retrospect it is clear that pH, $CO_2$ and alkalinity should have been monitored to ascertain if the inorganic fraction did increase.

Loss from the organic box could not be clearly ascribed to either dissolved or particulate forms. Possibly as bacteria reduced the carcasses of zooplankters and phytoplankters and bits of debris constituting the particulate matter, their (bacteria) biomass increases tended to offset decreases of the substrate. Both bacteria and detritus could have been added to dissolved carbon. $CO_2$ produced in bacterial respiration could be one route from organic to inorganic box.

Bacterial fixations of carbon dioxide is one possible route from inorganic to organic box. Further speculation about a dynamic system without further data seems fruitless. But within the conditions of this experiment, particulate organic fractions did not exhibit drastic decreases.

Table 27. Physical characteristics of waters sampled

| Water Body | Altitude m | Area ha | Max. Depth m | Mean Depth m | Remarks |
|---|---|---|---|---|---|
| Green Lake[1] | 3,520 | 1.4 | 7.0 | 3.2 | Cirque lake |
| Blue Lake[1] | 3,400 | 1.4 | 8.3 | 4.5 | Rock basin lake |
| Bear Lake[1] | 2,888 | 4.8 | 11.0 | 3.9 | Moraine dam lake |
| Cub Lake[1] | 2,629 | 3.5* | 4.0 | 2.0* | Brown water lake |
| Horsetooth Reservoir[2] | 1,656 | 755.0 | 62.0 | 24.0 | Fluctuates, data are maxima |
| Dixon Reservoir | 1,586 | 18.5 | 2.5 | 1.8* | Fluctuates, data are maxima |
| Glacier Creek | 2,806-2,562- | | | | Mountain brook, stony bed, rapid flow |
| Spring Creek | 1,586-1,500 | | | | Plains brook, sandy gravelly bed, slow flow |

1. Reed 1970

2. Nelson 1970

* estimated

Table 28. Concentrations of dissolved (DOC), net seston (NSC) and filter seston carbon (FSC) in Dixon Reservoir water during incubation after heating to 80C. Values for 29 April were obtained prior to heating. All values are in mg C/liter and most are means of duplicate samples

| Sampling Date | DOC | NSC | FSC |
|---|---|---|---|
| 29 Apr | 6.19 | .042 | .758 |
| 3 May | 4.46 | .030 | .524 |
| 5 | 5.98 | .043 | .632 |
| 10 | - | .028 | - |
| 12 | 5.18 | .027 | .520* |
| 14 | 5.05 | .032 | .652* |
| 17 | 5.90 | .052* | .640* |
| 19 | 5.12 | .038 | .532 |
| 21 | 5.05 | .033 | .700* |
| 24 | 5.20 | .039 | - |

*single determination

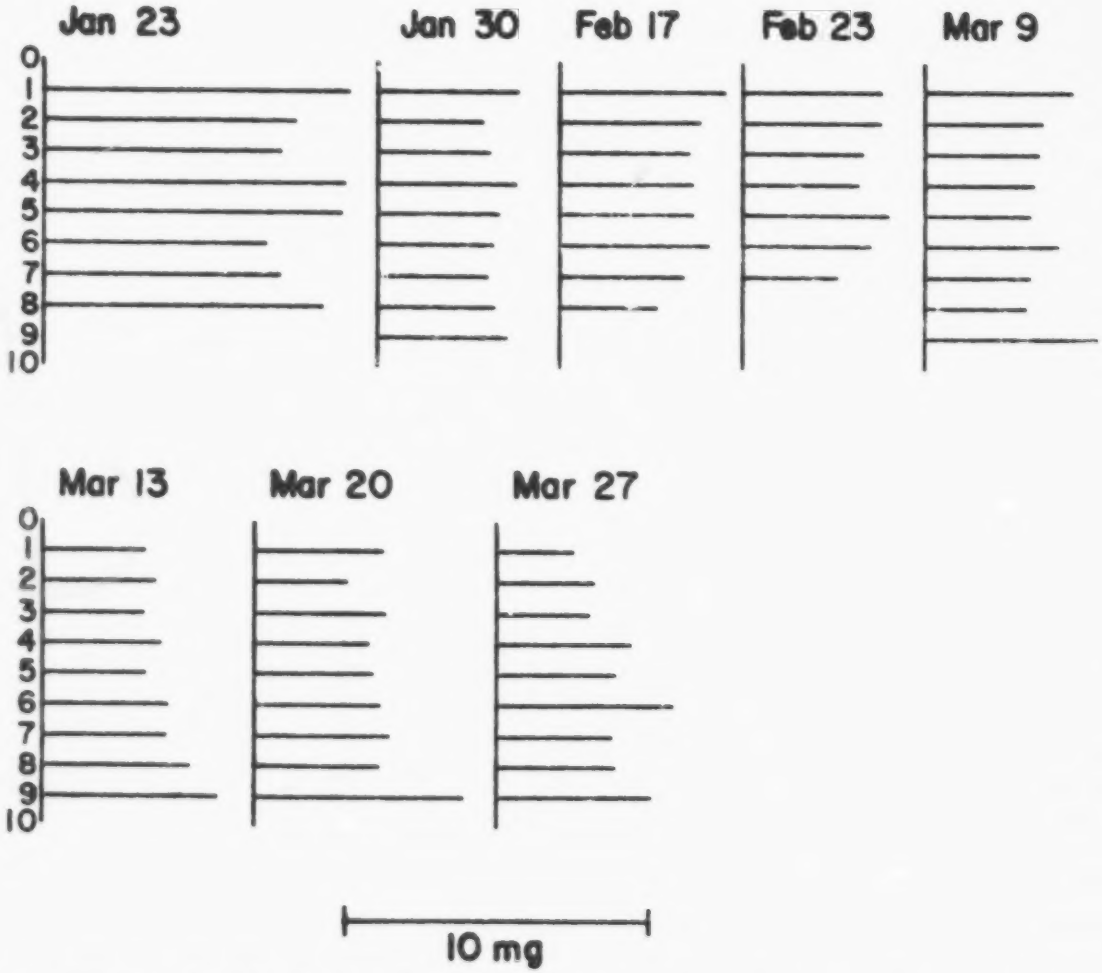


Figure 12. Vertical distribution of dissolved organic carbon in Bear Lake.

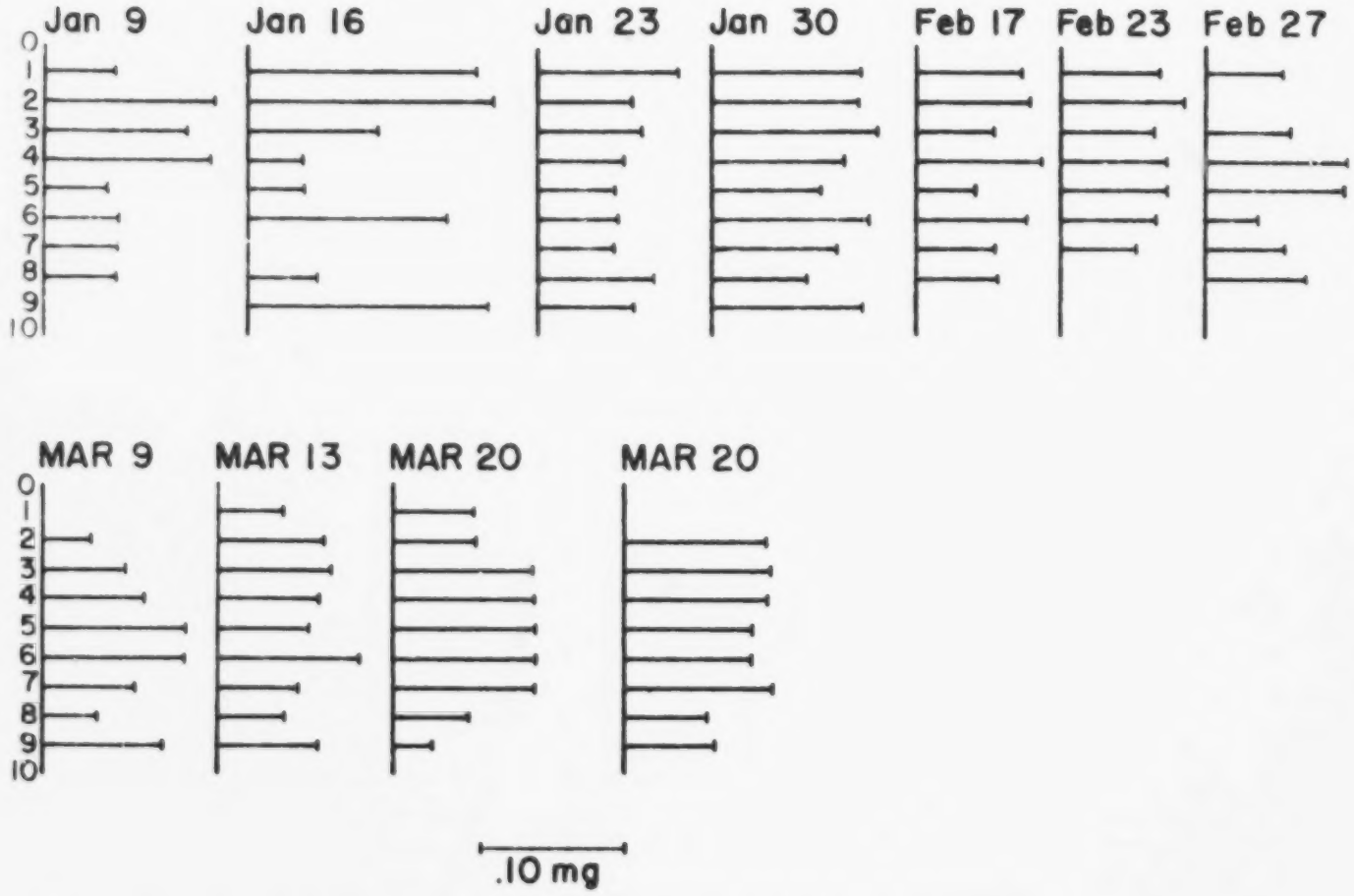


Figure 13. Vertical distribution of net seston carbon in Bear Lake.

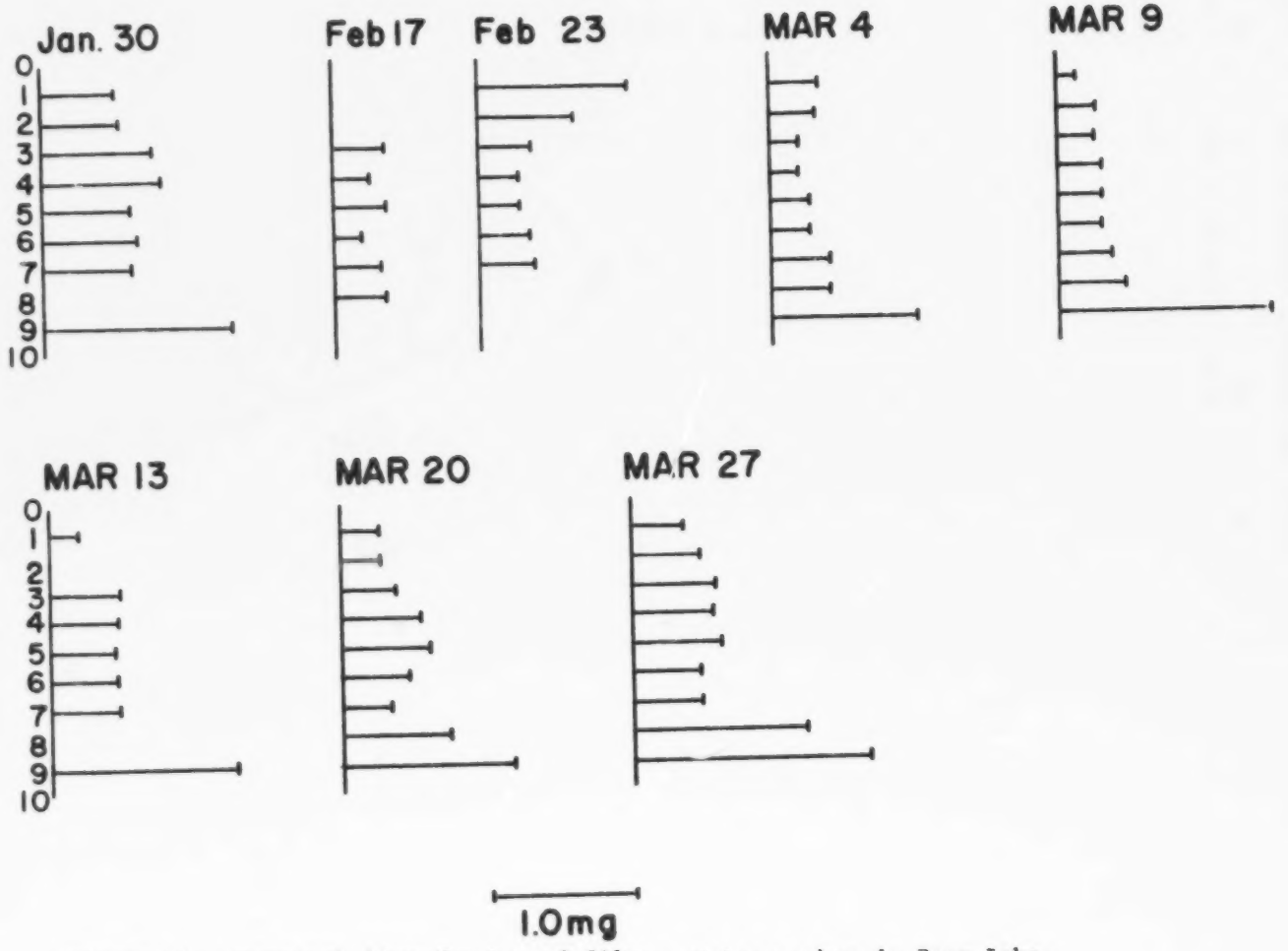


Figure 14. Vertical distribution of filter seston carbon in Bear Lake.

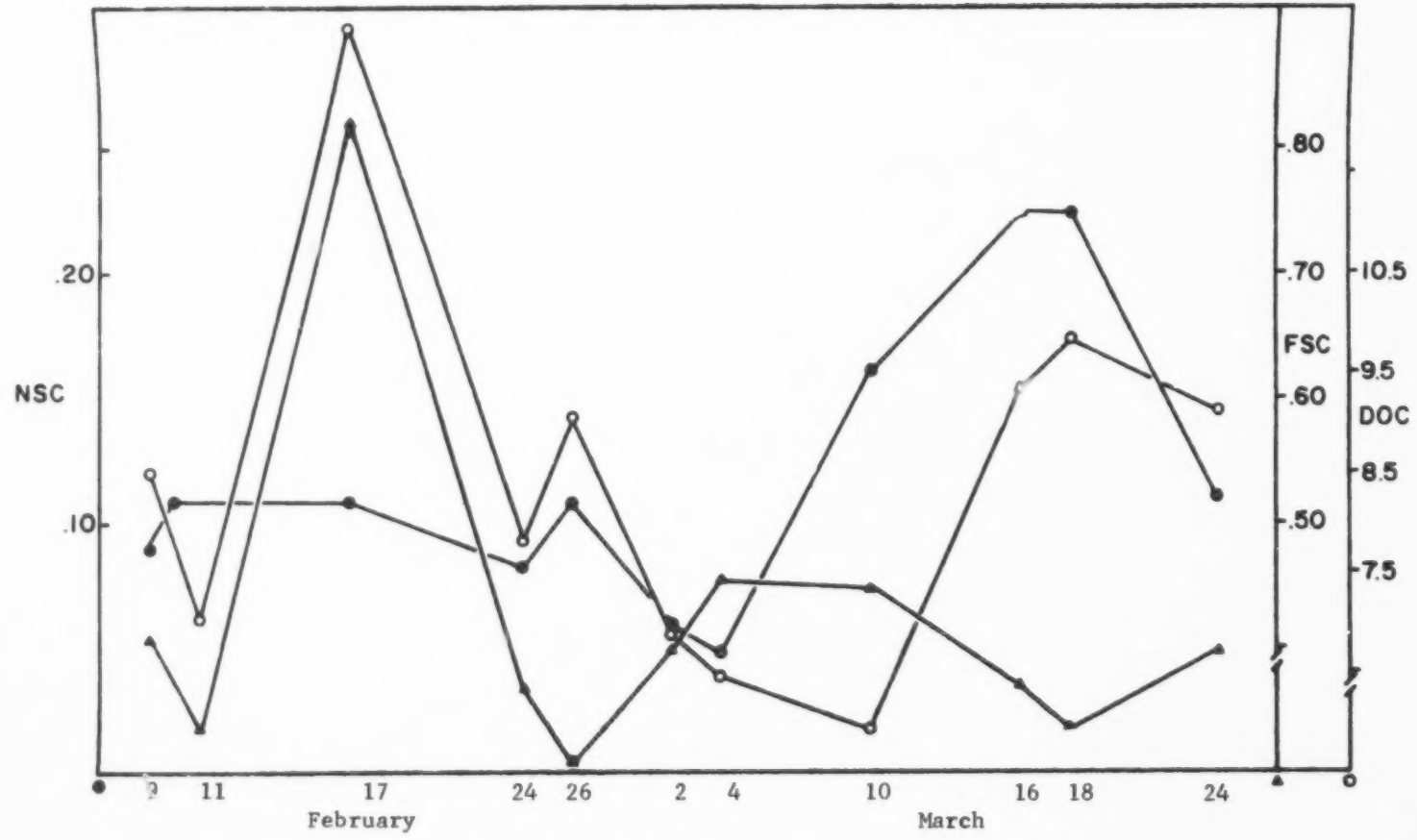


Figure 15. Dissolved organic carbon (triangles), filter seston carbon (open circles) and net seston carbon in Dixon Reservoir.

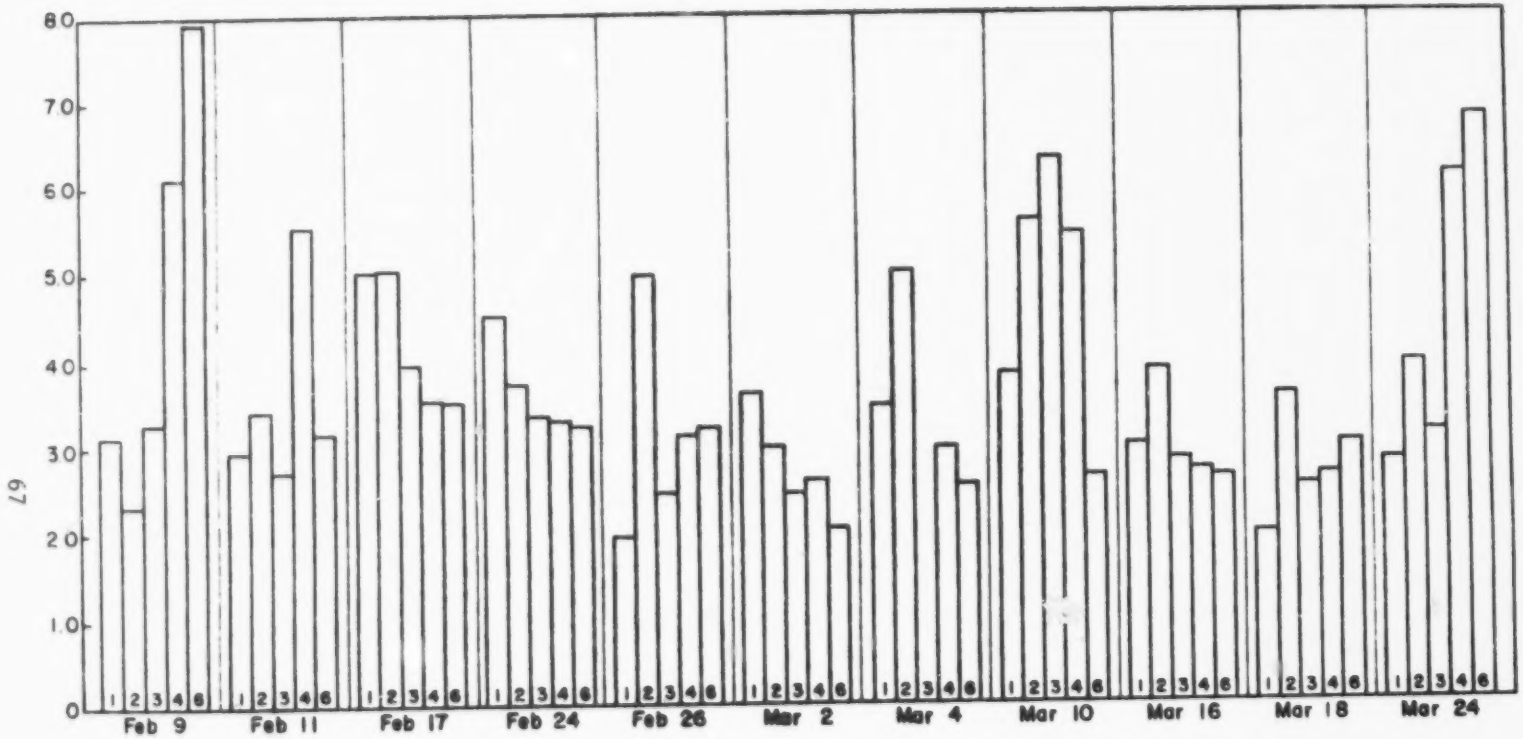


Figure 16. Dissolved organic carbon, four stations, Spring Creek.

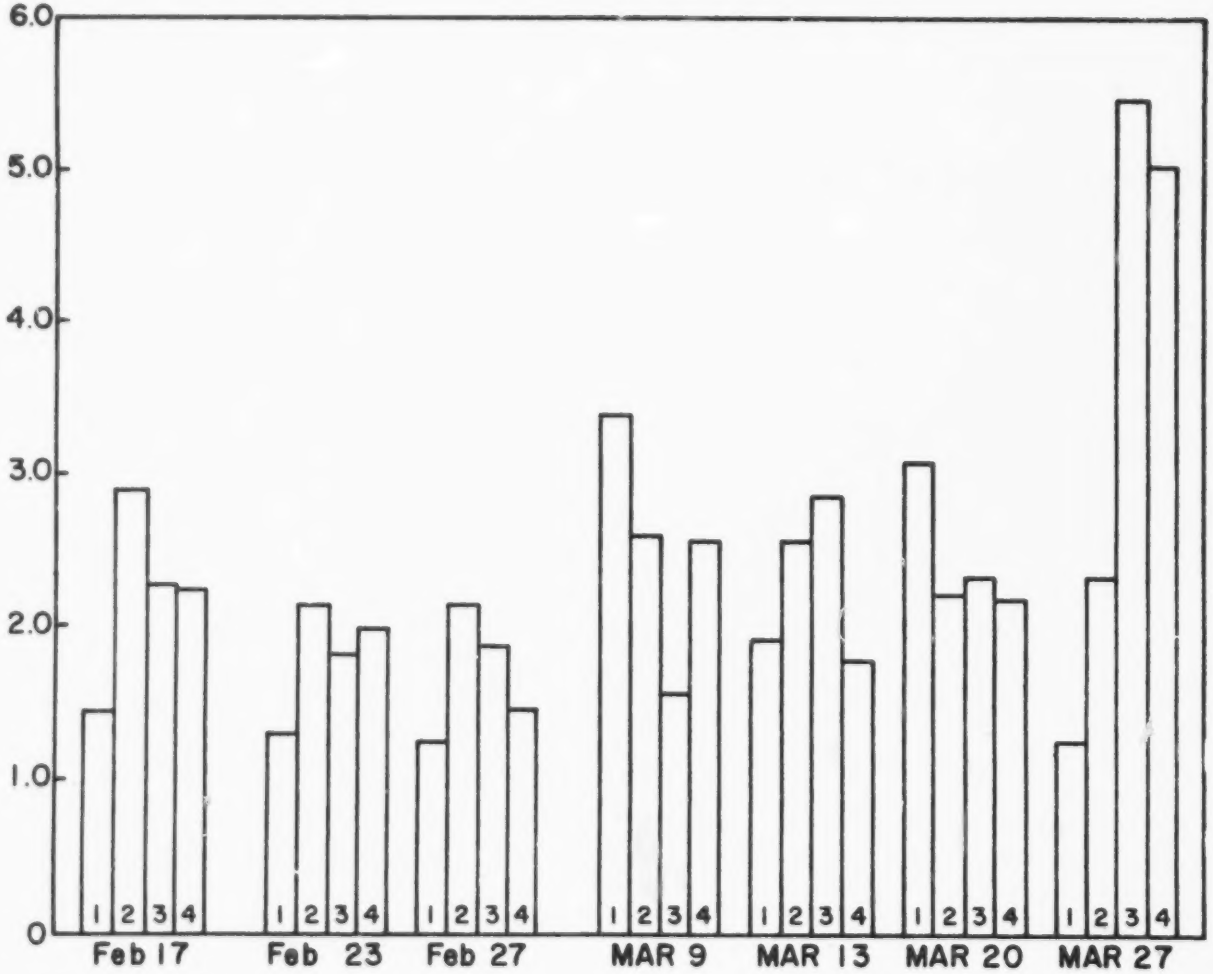


Figure 17. Dissolved organic carbon, four stations, Glacier Creek.

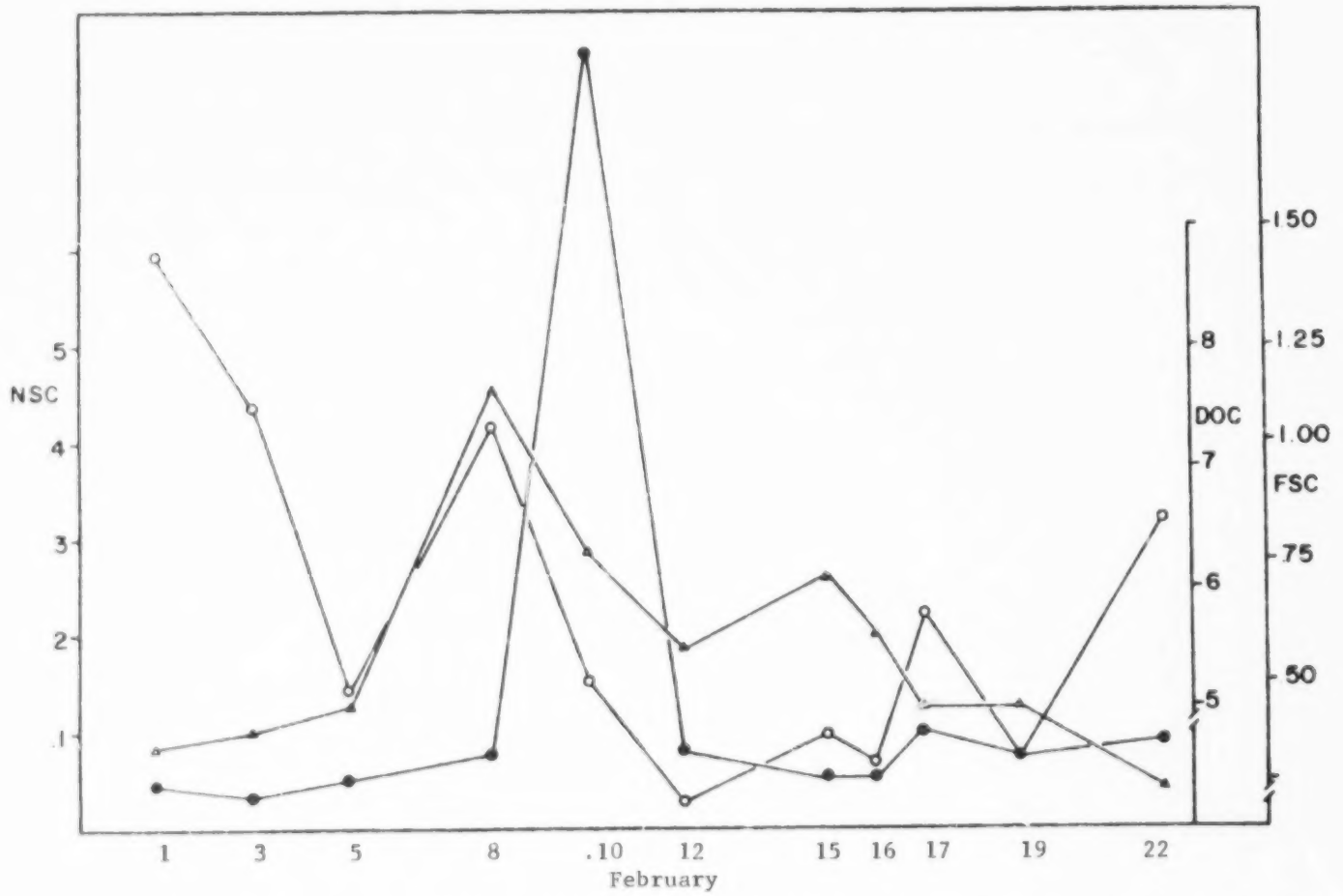


Figure 18. Changes in amounts of dissolved organic carbon (triangles), filter seston carbon (open circles) and net seston carbon from Dixon Reservoir during incubation (carboy experiment).

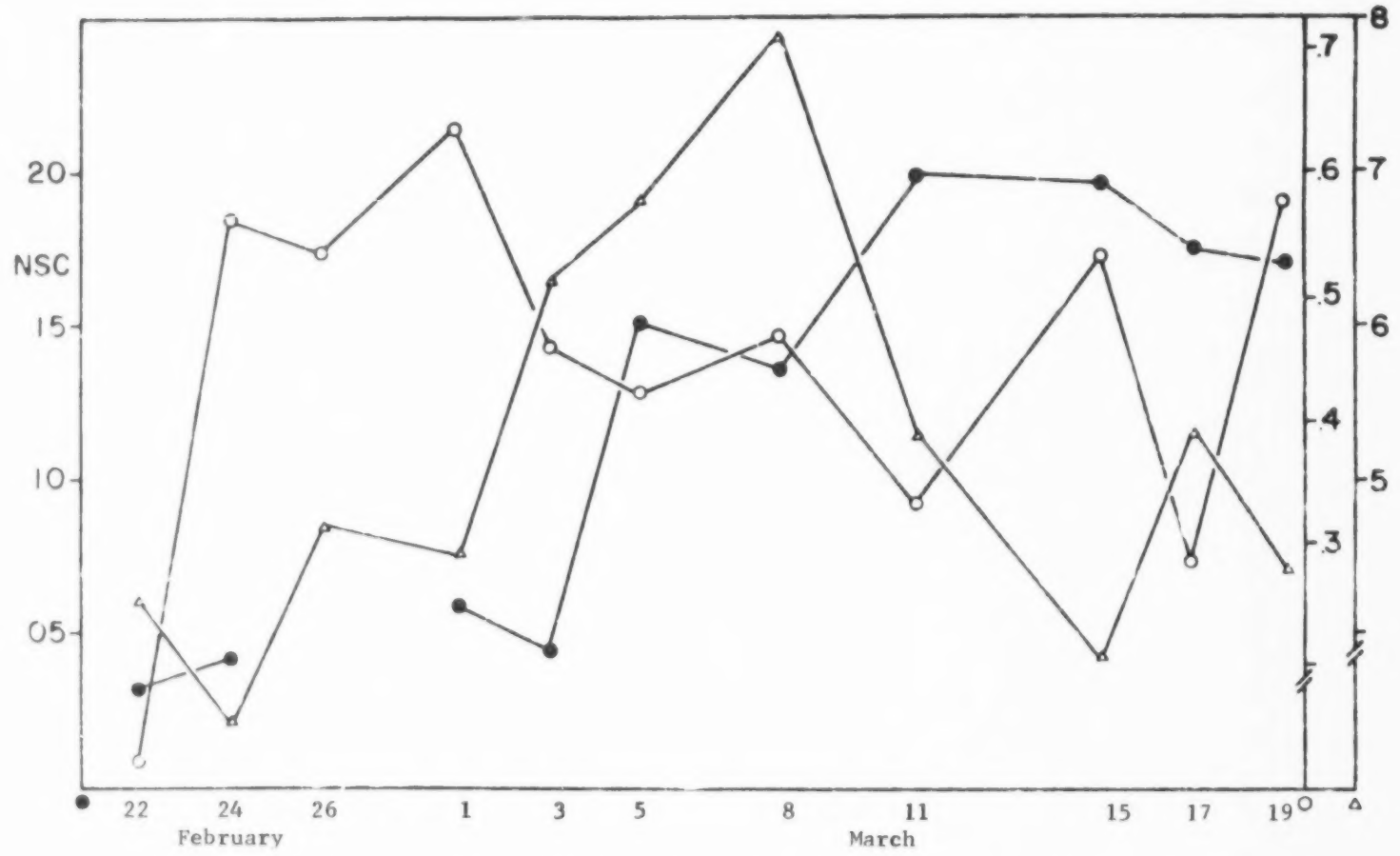


Figure 19. Changes in amounts of dissolved organic carbon (triangles), filter seston carbon (open circles) and net seston carbon from Dixon Reservoir during incubation (bottle experiment).

## SECTION VII

## DISCUSSION

Strickland and Parsons (1968) pointed out that while some theoretical doubt may exist, it is difficult to believe that the method of Menzel and Vaccaro fails to fully oxidize all organic compounds dissolved in sea water. Presumably the same is true for fresh water. Strickland and Parsons stated that the method is capable of detecting organic carbon in amounts as small as 0.09 mg/liter. As used in this study, the lower limit of detection appears to be about 1 mg C/liter or .005 mg C/5 ml ampoule, if the machine is calibrated so that 10 mg C/liter standard produced full scale deflection. In the case of NSC, if the carbonaceous material were strained from 12 liters of lake water, carbon was detected at the level of about .004 mg/liter in nature.

Strickland and Parsons suggested calibrating the analyzer so that 2 mg C/liter produced full scale deflection, thus giving a more sensitive setting than 10 mg C/liter standards. Calibration with 2.5 and 5 mg C/liter standards magnified noise and increased variability to undesirable levels.

Usually, a number of replicate samples analyzed at one time had an error ($100\ s\ /\ \bar{Y}$) of 10% or less. Thus, the errors associated with actual analysis were no greater than those associated with drawing the samples in nature.

One of the important pioneer studies of organic substances in fresh water was that of Birge and Juday (1934). They distinguished between particulate and dissolved forms of organic matter. Because they removed the particulate fraction by centrifugation, their findings cannot be compared with the net seston of this study. Their dissolved organic fraction should be roughly comparable to ours. The Wisconsin limnologists measured organic carbon in the surface waters of 529 lakes and found values ranging from 1.15 to 28.5 mg/liter. This range easily embraces the values reported here.

Using the method of Menzel and Vaccaro, Forsberg (1967) studied dissolved organic carbon in eight lakes of Uppland, Sweden. Forsberg designated some lakes as Chara lakes and others as Potamogeton lakes. In general, Chara lakes contained water showing from 10 to 55 mg color/liter (platinum - cobalt scale); whereas Potamogeton lakes had from 65 to 85 mg color/liter. Carbon values ranged from 5 to 14 mg/liter in Chara lakes and 11 to 14 mg/liter in Potamogeton lakes. The correlation of carbon with color was $r = 0.872$. The dominant macrophyte in Cub Lake is the pond lily, Nuphar polysepalum; the color of the water is, however, probably similar to that of the lower range of Forsberg's Chara lakes. Thus, the DOC values for Cub Lake, the lake in this survey with the most heavily stained water, are similar to values for brown-water Swedish lakes.

Forsberg made successive determinations of DOC in three lakes over a three-week period. Between 15 and 31 May, values of DOC in Ekoln varied from about 11 to 14 mg/liter. Greatest difference between two consecutive dates was 3 mg/liter. DOC values in Langsjon ranged from 5.2 to 6.8 mg/liter over the period 13 to 31 May. One mg/liter was the largest variation between successive readings. Concentrations of DOC ranged from 10.7 to 12.2 mg/liter in Trehorningen, a lake with much greater humus staining than Cub Lake. No common patterns to variation in DOC readings in the Swedish lakes were evident, although Forsberg noted that DOC and chlorophyll exhibited opposite tendencies, i.e. when DOC decreased, chlorophyll increased, supposedly indicating uptake of DOC by phytoplankton.

In a somewhat similar vein, Goldman and Armstrong (1969) apparently drew a number of subsamples at 24-hour intervals from lake and river probes for assay of C-14 uptake. Considerable variation among subsamples was found, but not discussed by these workers. Biologists have not been quick to elucidate short term changes in biological parameters of aquatic ecosystems, or for that matter, to document well their occurrences.

Concentrations of DOC in both Spring Creek and the Cache la Poudre River were roughly similar to values reported by Weber and Moore (1967) from the Little Miami River at Cincinnati. These investigators used a $CO_2$ infrared analysis method that differed from the one used in this study. DOC in the Little Miami ranged from 2.5 to 12.5 mg/liter and averaged 6.4. Fluctuations of 5 mg/liter occurred between adjacent weekly samplings. They concluded that DOC originated from several independent sources and thus could not be correlated with single parameters such as river discharge or standing crop of algae.

Some brief remarks regarding DOC and NSC in relation to other limnological variables can be made. In the summer of 1968, mean standing crops of seston in Green Lake were 3.51 $g/m^2$, Bear Lake 6.12 and Blue Lake 21.30 $g/m^2$ (Reed 1970). Stimpfl (1966) reported mean annual seston crops of 1.9 mg dry weight/liter and net seston crops of 1.969/$m^2$ for Horsetooth Reservoir, thus the lakes had the same relative rankings in dry weight of seston and DOC. In regard to NSC, Green, Blue and Bear lakes showed the same ranking as for DOC; Horsetooth by its variation and by being more frequently sampled could be placed on either end of the scale.

Vertical distribution of NSC and DOC in the lakes is difficult to summarize briefly. In general, maxima of both DOC and NSC at any one sampling tended to be found either in the upper layers or in the lower layers, but not at mid depth. Ohle (1968) reported that in nutrient rich lakes about 90% of the photosynthetic products of the epilimnion are broken down in the metalimnion. He further noted that destruction proceeds more slowly in the hypolimnion, but eventually increases at the mud-water interface. Overbeck (1968) found positive correlation between phytoplankton and bacteria maxima in lakes of east Holstein. He stated that heterotrophic bacteria depended on the phytoplankton for organic

substances, although bacteria and phytoplankton could exhibit different distributions in the water column. Maxima might occur together or bacterial maximum could occur in the hypolimnion simultaneously with a bloom of phytoplankton in the epilimnion.

Fonden (1969) noted that bacterial maxima tended to occur in or near the metalimnion in lake stations massively influenced by inflowing rivers. Away from inflow, maximum numbers of bacteria tended to be found in surface layers, or in any event, not in the hypolimnion. This is, of course, in agreement with Ohle's observations. Thus, apparent accumulation of carbon in deep water may not necessarily indicate the presence of large bacterial populations.

Nothing is known of bacterial populations associated with either DOC or NSC samples. It may be pointed out, however, that the simultaneous occurrence of phytoplankton and bacteria maxima does not necessarily imply a causal relationship. A bacterial maximum could be supported by a previous phytoplankton bloom at the time that another phytoplankton bloom developed. This could explain bacteria maximum in deep water occurring simultaneously with a pulse of phytoplankton in the epilimnion.

Nelson (1970) found that very little primary production, measured by C-14 uptake, occurred at depths greater than 6 m in Horsetooth Reservoir. Determinations of both DOC and NSC tended to agree with Nelson's findings. Support, or at least agreement, with Ohle's statement about retarded rates of decomposition in the hypolimnion may also be found in the Horsetooth results in that particulate organic matter easily attacked by bacteria could have been removed in the epilimnion, thus, accounting for lower concentrations of NSC below the metalimnion. However, the situation is undoubtedly more complex.

The nature of the particulate matter changed with depth in Horsetooth (and probably in most of the other lakes as well). Net seston samples taken below the metalimnion tended to be dominated by Daphnia and other relatively large zooplankters; whereas, epilimnion samples contained a preponderence of phytoplankters.

There seems to be no clear evidence from this study that deep water net seston in Horsetooth was more resistant to breakdown during incubation than was that from shallower depths. There is a weak suggestion that deep water net seston from Green and Blue lakes lost more carbon during incubation than did samples from shallower water. Results of incubation studies are inconclusive, but some trends are suggested. From 67 to 82% of the initial DOC values remained in Horsetooth water after incubation for 90 days. In one incubation series, 94% of the initial value of DOC was found in Blue Lake water after 90 days. In Bear, Green, and Cub Lakes, concentrations of DOC at the end of incubation were about 50% less than the initial concentration.

Values of NSC were more variable, but NSC in Horsetooth was apparently more resistant than that of any other body of water - 55 to 188% of

mean initial values was found after incubation. Readings of less than 10% of initial NSC were obtained for Bear, Blue, and Green Lakes.

No direct observations were made of bacteria, hence any remarks concerning them are completely speculative. However, one may assume that the organic matter, both particulate and dissolved, taken from these waters was complex and diverse in nature. Undoubtedly, a variety of bacteria were brought into the laboratory. It is not unreasonable to suppose that some were better suited to laboratory conditions than others. Vaccaro (1969) found that no single kinetic uptake pattern characterized the response of marine microplankton organisms in solutions enriched with specific organic substances. In natural waters a broad spectrum of organic substances is attacked by diverse assemblages of heterotrophic organisms. Vaccaro further pointed out that while results of short term (3 hour) uptake studies of heterotrophs in enriched media are unpredictable and erratic, irregular uptake patterns do tend to stabilize over time. This may be explained in part by the development of heterotrophic populations which are able to attack the specific substrate in question. Once the heterotroph population has become "adjusted" to the substrate (and in so doing changes from a mixed to essentially pure culture?) uptake follows predictable kinetic patterns.

The gain in carbon by samples during incubation requires comment. Because the gain was seen in many samples from different bodies of water and because gain was noted in series incubated and analyzed at different times, it cannot be dismissed simply as error. The following tentative explanation is suggested. Samples were incubated in darkness and covered to prevent entry of dust, but the aluminum caps over ampoules and reagent bottles were not airtight, thus $CO_2$ from the atmosphere could enter. Heterotrophic bacteria were able to attack organic matter contained in the samples for nutrients and at the same time fix carbon from the atmosphere. Ampoules and reagent bottles provided a large surface relative to the volume. The "surface effect" is well recognized in C-14 uptake studies and in BOD determinations; in essence, the glass surface provides a stable substrate upon which bacteria can attach and multiply.

Ultimately, the bacteria exhaust some nutrient to a limiting level or perhaps their metabolic products reach intolerable levels, but in any event, the population cannot grow continuously in a limited environment. A rapid decline ensues and the bacteria population either dies out or fluctuates about some low level.

Probably some sorting out of species also occurs and any given sample becomes dominated by one or a few species whose metabolic processes are complimentary. If a sample is analyzed when a large bacterial population is fixing atmospheric carbon, carbon value in excess of the initial content is possible.

Kuznetsov (1968) emphasized the importance of heterotrophic bacteria in production dynamics of certain European lakes and reservoirs.

Heterotrophic assimilation of carbonic acid accounted for 24% of total organic matter produced in Lake Onega and in Rybinsk Reservoir production of bacterial biomass exceeded photoplankton biomass production in 1964. Rybinsk Reservoir apparently receives massive amounts of allochthonous organic detritus. The detritus is broken down by bacteria, releasing carbon dioxide which is utilized by heterotrophic bacteria and photoplankters.

The major thesis, that relative fractions of refractive and non-refractive organic carbon, either particulate or in solution, in freshwaters might offer clues as to trophic state remains neither strongly endorsed nor denied by this investigation.

In the increased acuity of vision that comes with hindsight, the original concepts of incubation studies appear naive, to say the least. The results reported are not without value, although neat, clear-cut conclusions are not to be drawn. The incubation studies are, of course, a variation on the classic BOD determinations, as seen from the viewpoint of carbon rather than oxygen. Because of heterotrophic bacteria (and algae?) the carbon story may be much more complicated than the tale told by oxygen. Certainly, the results suggest that smooth decay curves cannot describe the events occurring in the incubation studies.

In regard to natural waters, the findings indicate that organic carbon concentrations, both dissolved and particulate, can and do fluctuate markedly over short intervals of time. Flowing waters have long been recognized to be more variable environments than lentic waters, thus, the demonstration the DOC fluctuates with time in small streams can hardly be considered astounding.

Few studies have followed changes of organic carbon for several weeks in one body of water. The data thus have descriptive value, although they cannot, at this time, be related satisfactorily to the body of general limnological knowledge. One might suppose that carbon contained in a population of plankters would eventually find its way from large particulate chunks through fine particles to the dissolved state. Of course, it is well recognized that healthy photoplankters release considerable amounts of photosynthetically fixed carbon in their metabolic processes. Inputs of carbon to the plankton community from littoral, benthic and allochthonous sources offer opportunities for rapid changes in organic carbon. Further complications arise from exchanges between inorganic and organic fractions of carbon.

In view of its possible role as a limiting nutrient (Kerr, Paris and Brockway 1970 ?) in some aquatic ecosystems, carbon deserves further study. A number of papers presented at the annual meeting of the American Society of Limnology and Oceanography, University of Rhode Island (August 1970) and at the Symposium on Nutrients and Eutrophication, sponsored by the same society at W. K. Kellogg Biological Station (February 1971) referred to organic carbon. Reported values ranged over at

least two orders of magnitude. Seemingly much work simply describing instantaneous amounts of organic carbon remains to be done.

## SECTION VIII

## ACKNOWLEDGMENTS

The support of the project by the Water Quality Office, Environmental Protection Agency and the help provided by Dr. L. P. Seyb, the Grant Project Officer, is acknowledged with sincere thanks.

## SECTION IX

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5 Organization

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Department of Zoology

6 Title

Dissolved and Particulate Organic Carbon in Some Colorado Waters

10 Author(s)

Reed, Edward B.

16 Project Designation

EQA 16010

21 Note

22 Citation

23 Descriptors (Starred First)

*Lakes, carbon *Reservoirs, carbon #Nutrients, carbon

25 Identifiers (Starred First)

*Carbon, organic

27 Abstract

Organic carbon in a variety of lakes, reservoirs and streams was measured by acid-persulfate digestion and infrared absorption.

Organic carbon was designated as net seston carbon, filter seston carbon or dissolved based on filtering techniques.

Concentrations of dissolved carbon ranged over an order of magnitude; i.e. from about 1 to 11 $g/m^3$; filter seston amounts varied from about p.2 to 1 $g/m^3$; the net fraction of organic carbon ranged from less than 0.1 to over 0.2 $g/m^3$. In general bodies of water judged to be meso- to eutrophic contained more organic carbon than those waters tending toward oligotrophy.

Repeated sampling of selected bodies of water revealed that amounts of organic carbon, either dissolved or particulate, fluctuated considerably over short periods of time; however the dissolved fraction probably varied less than particulate forms of carbon.

Samples of all carbon fractions were incubated at room temperature in darkness for periods ranging up to 90 days. Concentrations of organic carbon varied erratically and unpredictably during incubation.

Almost daily measurements of carbon in samples incubated over three week periods also revealed erratic changes, with no clear reduction in total organic carbon. Metabolic activities of heterotrophic bacteria (and algae?) probably complicated interpretation of results.

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